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(57) Abstract

The present invention relates to a novel glial cell line-derived neurotrophic factor receptor beta (GDNFR-β) and novel glial cell line-derived neurotrophic factor receptor gamma 1 and 2 (GDNFR-γ1 and GDNFR-γ2). The receptors of the present invention share high homology with glial cell line-derived neurotrophic factor receptor alpha and have been named GDNFR-β, GDNFR-γ1 and GDNFR-γ2. More specifically, isolated nucleic acid molecules are provided encoding human GDNFR-β, GDNFR-γ1 and GDNFR-γ2 receptors. GDNFR-β, GDNFR-γ1 and GDNFR-γ2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of GDNFR-β, GDNFR-γ1 and GDNFR-γ2 activity. Also provided are diagnostic and therapeutic methods for disorders and diseases including Parkinson's disease, thyroid tumor, kidney failure and gut dysfunction.

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GDNF Receptors

Background of the Invention

Field of the Invention

The present invention relates to a novel glial cell line-derived neurotrophic factor receptor beta (GDNFR-β) and novel glial cell line-derived neurotrophic factor receptor gamma 1 and 2 (GDNFR-γ1 and GDNFR-γ2). The receptors of the present invention share high homology with glial cell line-derived neurotrophic factor receptor alpha and have been named GDNFR-β, GDNFR-γ1 and GDNFR-γ2. More specifically, isolated nucleic acid molecules are provided encoding human GDNFR-β, GDNFR-γ1 and GDNFR-γ2 receptors. GDNFR-β, GDNFR-γ1 and GDNFR-γ2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of GDNFR-β, GDNFR-γ1 and GDNFR-γ2 activity. Also provided are diagnostic and therapeutic methods for disorders and diseases including Parkinson's disease, thyroid tumor, kidney failure and gut dysfunction.

Related Art

Glial cell line-derived neurotrophic factor (GDNF) was first characterized as a potent neurotrophic factor that enhances the survival of midbrain dopaminergic neurons (Lin *et al., Science 260*:1130-1132 (1993)). Further, studies have expanded the functional role of GDNF to include the protection of dopaminergic neurons from degeneration *in vitro* and the improvement of Parkinson's disease-like symptoms in animal models (Beck *et al. Nature 373*:339-341 (1995); Hou *et al. J. Neurochem 66*: 74-82 (1996)). Studies examining expression patterns of GDNF have observed expression in several additional classes of peripheral neurons and in muscle (Trupp *et al. J. Cell Biol. 130*:137-148 (1995)). In addition, renal abnormalities (kidney agenesis or dysgenesis) and enteric neuronal abnormalities are observed in transgenic mice lacking GDNF

(Pichel, *et al. Nature 382*:73-75 (1996)). This study demonstrated that GDNF induces ureter bud formation and branching during metanephros developmet and is essential for proper innervation of the gastrointestinal tract (Pichel, *et al. Nature 382*:73-75 (1996)).

The search for the GDNF receptor molecule has recently resulted in the molecular cloning of a rat and a human GDNF receptor-α (Jing *et al., Cell 85*: 1113-1124 (1996); Treanor *et al., Nature 382*:80-83 (1996)). Two independent groups identified the first component of the GDNF receptor, designated GDNFR-α. Studies on GDNFR-α revealed that this receptor does not contain a cytoplasmic component, rather it is anchored to the plasma membrane via a glycosylphosphotidylinositol linkage (Jing *et al., Cell 85*: 1113-1124 (1996); Treanor *et al., Nature 382*:80-83 (1996)). Much like the cytokine receptor system, GDNFR-α is only a single component of the functional receptor for GDNF. The signaling component of the functional receptor for GDNF was determined to be an orphan receptor tyrosine kinase designated *Ret* (Jing *et al., Cell 85*: 1113-1124 (1996); Treanor *et al., Nature 382*:80-83 (1996); Trupp et al., Nature 381:785-789 (1996)). Surprisingly, the affinity of the *Ret*/GDNFR-α receptor complex was not found to be significantly higher than for GDNFR-α alone (Jing *et al., Cell 85*: 1113-1124 (1996); Treanor *et al., Nature 382*:80-83 (1996); Trupp *et al., Nature 381*:785-789 (1996)). As a result, these findings suggest the presence of additional homologues of either, or both, *Ret* or GDNFR-α.

Studies that have analyzed the effects of defective GDNF expression in mice, have identified such disease states as Parkinson's disease, thyroid tumor, kidney failure and gut dysfunction as possibly being associated with the lack of a functional GDNF pathway (Jing *et al., Cell 85*: 1113-1124 (1996); Trupp et al., Nature 381:785-789 (1996) Angrist *et al. Nat. Genet 14*:341-344 (1996); Vega *et al., Proc. Natl. Acad. Sci. 93*:10657-10661 (1996)). Studies in *in vivo* models for Parkinson's disease have shown that administration of GDNF dramatically corrects motor deficits which has prompted the intiation of GDNF clinical trials.

Thus, novel homologues of either component of the GDNF receptor complex could prove useful in discovering pharmacologically valuable factors for regulating the complex processes of neuronal function. Clearly there is a need for identification and characterization of further GDNF receptor polypeptides which can play a role in diagnosing preventing, ameliorating or correcting dysfunctions or diseases, including Parkinson's disease, thyroid tumor, kidney failure and gut dysfunction.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel GDNFR-β receptor having the amino acid sequence shown in Figure 1 (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 97883 on February 14, 1997. The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of GDNFR-β polypeptides or peptides by recombinant techniques. The invention further provides an isolated GDNFR-β polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel GDNFR-γ1 receptor having the amino acid sequence shown in Figure 4 (SEQ ID NO:5) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 209051 on May 16, 1997. The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of GDNFR-γ1 polypeptides or peptides by recombinant techniques.

The invention further provides an isolated GDNFR-γ1 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention further provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel GDNFR-γ2 receptor having the amino acid sequence shown in Figure 7 (SEQ ID NO:7) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 209052 on May 16, 1997. The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of GDNFR-γ2 polypeptides or peptides by recombinant techniques. The invention further provides an isolated GDNFR-γ2 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor, which involves contacting cells which express the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor with the candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a screening assay for agonists and antagonists is provided which involves determining the effect a candidate compound has on GDNF binding to the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor. In particular, the method involves contacting the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor with a GDNF polypeptide and a candidate compound and determining whether GDNF polypeptide binding to the GDNFR-β GDNFR-γ1

or GDNFR-γ2 receptor is increased or decreased due to the presence of the candidate compound.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide of the invention or an agonist thereof.

A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an GDNFR-β, GDNFR-γ1 or GDNFR-γ2 antagonist.

The invention further provides a diagnostic method useful during diagnosis or prognosis of diseases and disorders including Parkinson's disease, schizophrenia, insomnia, tardive dyskenisia, hypertension, pituitary adenomas, hyperprolactinemia, thyroid tumor, renal disorders, kidney failure and gut dysfunction.

Brief Description of the Figures

Figure 1 shows the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of GDNFR-β receptor. The protein has a deduced molecular weight of about 53 kDa for the non-glycosylated form. The protein has several potential glycosylation sites (N-linked). The predicted molecular weight of the cell surface bound receptor is around 75 kDa. The protein has a predicted leader sequence of about 21 amino acid residues (underlined). It is further predicted that amino acid residues from about 22 to about 448 constitute the extracellular domain; and from about 449 to about 464 the transmembrane domain.

Figure 2 shows the regions of similarity between the amino acid sequences of the GDNFR-β receptor protein and the rat GDNFR-α (SEQ ID NO:3).

Figure 3 shows an analysis of the GDNFR-β receptor amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 23-80, 89-106, 119-137, 149-221, 226-291, 310-395 and 432-449 in Figure 1 (or amino acid residues 2-59, 68-85, 98-116, 128-200, 205-270, 289-374, and 411-428 in SEQ ID NO:2) correspond to the shown highly antigenic regions of the GDNFR-β receptor protein.

Figure 4 shows the nucleotide (SEQ ID NO:4) and deduced amino acid (SEQ ID NO:5) sequences of GDNRF-γ1 receptor. The protein has a deduced molecular weight of about 42 kDa for the non-glycosylated form. The protein has a predicted leader sequence of about 31 amino acid residues (underlined). It is further predicted that amino acid residues from about 32 to about 360 constitute the extracellular domain; and from about 361 to about 378 the transmembrane domain.

Figure 5 shows the regions of similarity between the amino acid sequences of the GDNFR-γ1 receptor protein and the rat GDNFR-α (SEQ ID NO:3).

Figure 6 shows an analysis of the mature GDNFR-γ1 receptor amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 2-10, 13-26, 33-40, 42-56, 59-67, 71-77, 90-114, 122-129, 139-164, 174-180, 187-203, 217-235, 250-257, 302-307, and 317-325 in SEQ ID NO:5 correspond to the shown highly antigenic regions of the mature GDNFR-γ receptor protein.

Figure 7 shows the nucleotide (SEQ ID NO:6) and deduced amino acid (SEQ ID NO:7) sequences of GDNFR-γ2 receptor. The protein has a deduced molecular weight of about 45 kDa for the non-glycosylated form. The protein has

a predicted leader sequence of about 31 amino acid residues (underlined). It is further predicted that amino acid residues from about 32 to about 382 constitute the extracellular domain; and from about 383 to about 400 the transmembrane domain.

Figure 8 shows the regions of similarity between the amino acid sequences of the GDNFR-γ2 receptor protein and the rat GDNFR-α (SEQ ID NO:3).

Detailed Description

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel GDNFR-β polypeptide having the amino acid sequence shown in Figure 1 (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The GDNFR-β protein of the present invention shares sequence homology with rat GDNFR-α (Figure 2) (SEQ ID NO:3). The nucleotide sequence shown in Figure 1 (SEQ ID NO:1) was obtained by sequencing a cDNA clone (HSSAE30), which was deposited on February 14, 1997 at the American Type Culture Collection, Patent Depository, 10801 University Boulevard, Manassas, Virginia, 20110-2209, and given accession number 97883. The cDNA was inserted between the BamHI and Asp718 in the plasmid pC4.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel GDNFR-γ1 polypeptide having the amino acid sequence shown in Figure 4 (SEQ ID NO:5), which was determined by sequencing a cloned cDNA. The GDNFR-γ1 protein of the present invention shares sequence homology with rat GDNFR-α (Figure 2) (SEQ ID NO:3). The nucleotide sequence shown in Figure 4 (SEQ ID NO:4) was obtained by sequencing a cDNA clone (HETDK21X), which was deposited on May 16, 1997 at the American Type Culture Collection, Patent Depository, 10801 University Boulevard, Manassas, Virginia, 20110-2209, and given accession number 209051.

The cDNA was inserted between the EcoRI and XhoI in the polylinker of the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

The present invention further provides isolated nucleic acid molecules comprising a polynucleotide encoding a novel GDNFR-γ2 polypeptide having the amino acid sequence shown in Figure 7 (SEQ ID NO:7), which was determined by sequencing a cloned cDNA. The GDNFR-γ2 protein of the present invention shares sequence homology with rat GDNFR-α (Figure 2) (SEQ ID NO:3). The nucleotide sequence shown in Figure 7 (SEQ ID NO:7) was obtained by sequencing a cDNA clone (HETDK21501XX), which was deposited on May 16, 1997 at the American Type Culture Collection, Patent Depository, 10801 University Boulevard, Manassas, Virginia, 20110-2209, and given accession number 209052. The cDNA was inserted between the EcoRI and XhoI sites in the polylinker of the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined

nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in Figures 1, 4, or 7 a nucleic acid molecule of the present invention encoding a GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide, respectively, may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figure 1 (SEQ ID NO:1) was discovered in a cDNA library derived from human synovial sarcoma. The gene was also identified in cDNA libraries from spleen (from a chronic lympocytic leukemia patient), adult testis, infant brain, primary dendric cells, among others. The determined nucleotide sequence of the GDNFR-β cDNA of Figure 1 (SEQ ID NO:1) contains an open reading frame encoding a protein of about 464 amino acid residues, with a predicted leader sequence of about 21 amino acid residues, and a deduced molecular weight of about 53 kDa for the non-glycosylated form. The amino acid sequence of the predicted mature GDNFR-β receptor is shown in Figure 1 from amino acid residue about 22 to residue about 464. The GDNFR-β protein shown in Figure 1 (SEQ ID NO:2) is about 45 % identical and about 60 % similar to rat GDNFR-α (Figure 2).

Also illustrative of the invention is the nucleic acid molecule described in Figure 4 (SEQ ID NO:4) was discovered in a cDNA library derived from human endometrial tumor. The gene was also identified in cDNA libraries from the following tissues: fetal epithelium, fetal heart, fetal liver and nine week old early stage human embryos. The determined nucleotide sequence of the Figure 4 (SEQ ID NO:4) contains an open reading frame encoding a protein of about 378 amino acid residues, with a predicted leader sequence of about 31 amino acid residues, and a deduced molecular weight of about 42 kDa. The GDNFR-γ1 cDNA of clone HETDK21 does not represent the full-length coding sequence of GDNFR-γ. However, a 5'-RACE-based experiment was successful in isolating an additional

163 bp of cDNA sequence 5' of the start of clone HETDK21 from a human endometrial tumor cDNA library. This additional sequence fragment appears to contain the start Methionine and a signal sequence for human GDNFR-γ1. This additional 163 bp of cDNA sequence has been merged below with the sequence contained in the HETDK21 clone to yield the full-length sequence for GDNFR-γ1 as shown in Figure 4 (SEQ ID NO:4). The GDNFR-γ1 protein shown in Figure 4 (SEQ ID NO:5) is about 34.3 % identical and about 51% similar to rat GDNFR-α (Figure 5).

Also illustrative of the invention is the nucleic acid molecule described in Figure 7 (SEQ ID NO:7) was discovered in a cDNA library derived from human endometrial tumor. The gene was also identified in cDNA libraries from the following tissues: fetal epithelium, fetal heart, fetal liver and nine week old early stage human embryos. The determined nucleotide sequence of the Figure 7 (SEQ ID NO:7) contains an open reading frame encoding a protein of about 400 amino acid residues, with a predicted leader sequence of about 31 amino acid residues, and a deduced molecular weight of about 45 kDa. The GDNFR-γ2 protein shown in Figure 7 (SEQ ID NO:7) is about 34.4 % identical and about 50.6% similar to rat GDNFR-α (Figure 5).

As indicated, the present invention also provides the mature form(s) of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature GDNFR-β

polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 97883 and as shown in Figure 1 (SEQ ID NO:2). The present invention also provides a nucleotide sequence encoding the mature GDNFR-γ1 polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 209051 and as shown in Figure 4 (SEQ ID NO:5). The present invention also provides a nucleotide sequence encoding the mature GDNFR-γ2 polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 209052 and as shown in Figure 7 (SEQ ID NO:7). By the mature GDNFR-β protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit 97883 is meant the mature form(s) of the GDNFR-β receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. By the mature GDNFR-γ1 protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit 209051 is meant the mature form(s) of the GDNFR-γ1 receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. By the mature GDNFR-γ2 protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit 209052 is meant the mature form(s) of the GDNFR-γ2 receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature GDNFR-β receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883 may or may not differ from the predicted "mature"GDNFR-β protein shown in Figure 1 (amino acids from about 22 to about 464) depending on the accuracy of the predicted cleavage site based on computer analysis. The mature

GDNFR-γ1 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051 may or may not differ from the predicted "mature" GDNFR-γ1 protein shown in Figure 4 (amino acids from about 32 to about 378) depending on the accuracy of the predicted cleavage site based on computer analysis. The mature GDNFR-γ2 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052 may or may not differ from the predicted "mature" GDNFR-γ2 protein shown in Figure 7 (amino acids from about 32 to about 400) depending on the accuracy of the predicted cleavage site based on computer analysis.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the methods of McGeoch (*Virus Res. 3*:271-286 (1985)) and von Heinje (*Nucleic Acids Res. 14*:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the predicted amino acid sequence of the complete GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide of the present invention were analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 21 and 22 in Figure 1 (amino acids -1 and 1 in SEQ ID NO:2); a cleavage site between amino acids 31 and 32 in Figure 4 (amino acids -1 and 1 in SEQ ID NO:5); and a cleavage site between amino acids 31 and 32 in Figure 7 (amino acids -1 and 1 in SEQ ID NO:7). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heine. von Heinje, *supra*. Thus, the leader sequence for the GDNFR-β receptor protein is predicted to consist of amino acid residues 1-21

in Figure 1 (amino acids -21 to -1 in SEQ ID NO:2) , while the predicted mature GDNFR-β protein consists of residues 22-464 (amino acids 1 to 443 in SEQ ID NO:2). The leader sequence for the GDNFR-γ1 receptor protein is predicted to consist of amino acid residues 1-31 in Figure 4 (amino acids -31 to -1 in SEQ ID NO:5), while the predicted mature GDNFR-γ1 protein consists of residues 32-378 (amino acids 1 to 347 in SEQ ID NO:5). The leader sequence for the GDNFR-γ2 receptor protein is predicted to consist of amino acid residues 1-31 in Figure 7 (amino acids -31 to -1 in SEQ ID NO:7), while the predicted mature GDNFR-γ2 protein consists of residues 32-400 (amino acids 1 to 369 in SEQ ID NO:7).

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

As one of ordinary skill would appreciate, however, due to the possibilities of sequencing errors, the GDNFR-β receptor polypeptide encoded by the deposited cDNA comprises about 464 amino acids, but may be anywhere in the range of 450-480 amino acids; and the leader sequence of this protein is about 21 amino acids, but may be anywhere in the range of about 10 to about 30 amino acids. As one of ordinary skill would also appreciate, however, due to the possibilities of sequencing errors, the GDNFR-γ1 receptor polypeptide encoded by the deposited cDNA comprises about 378 amino acids, but may be anywhere in the range of 350-400 amino acids; and the leader sequence of this protein is about 31 amino acids, but may be anywhere in the range of about 20 to about 40 amino acids. As one of ordinary skill would also appreciate, however, due to the possibilities of sequencing errors, the GDNFR-γ1 receptor polypeptide encoded by the deposited cDNA comprises about 378 amino acids, but may be anywhere in the range of 350-400 amino acids; and the leader sequence of this protein is about 31 amino acids, but may be anywhere in the range of about 20 to about 40

amino acids. As one of ordinary skill would also appreciate, however, due to the possibilities of sequencing errors, the GDNFR-γ2 receptor polypeptide encoded by the deposited cDNA comprises about 400 amino acids, but may be anywhere in the range of 380-420 amino acids; and the leader sequence of this protein is about 31 amino acids, but may be anywhere in the range of about 20 to about 40 amino acids.

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 which have been determined from the following related cDNA clones: HSSAE30R (SEQ ID NO: 8), HTLBC22R (SEQ ID NO: 9), HIBCK30R (SEQ ID NO: 10), R02249.nt (SEQ ID NO: 11), H12981.nt (SEQ ID NO: 12), W73681.nt (SEQ ID NO: 13), W73633.nt (SEQ ID NO: 14), H05619.nt (SEQ ID NO: 15), R02135.nt (SEQ ID NO: 16), T03342.nt (SEQ ID NO: 17), and Z43761.nt (SEQ ID NO: 18).

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO: 4 (GDNFR-γ1) which have been determined from the following related cDNA clones: HETDK21R (SEQ ID NO: 19), HFLQD75R (SEQ ID NO:20), W69774.nt (SEQ ID NO:21), W69813.nt (SEQ IDNO:22).

Further, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO: 6 (GDNFR-γ2) which have been determined from the following related cDNA clones: HETDK21R (SEQ ID NO: 19), HFLQD75R (SEQ ID NO:20), W69774.nt (SEQ ID NO:21), W69813.nt (SEQ IDNO:22).

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:6); DNA molecules comprising the coding sequence for the GDNFR-β receptor shown in Figure 1 (SEQ ID NO:2), the GDNFR-γ1 receptor shown Figure 4 (SEQ ID NO:5) or the GDNFR-γ2 receptor shown Figure 7 (SEQ ID NO:7); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the GDNFR-β, the GDNFR-γ1 or the GDNFR-γ2 receptor. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the GDNFR-β polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97883 on February 14, 1997. Another aspect, the invention provides isolated nucleic acid molecules encoding the GDNFR-γ1 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 209051 on May 16, 1997. A further aspect, the invention provides isolated nucleic acid molecules encoding the GDNFR-γ2 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 209052 on May 16, 1997. Preferably,

these nucleic acid molecules will encode the mature polypeptides encoded by the above-described deposited cDNA clones. In a further emodiment, nucleic acid molecules are provided encoding the GDNFR-β, the GDNFR-γ1 or the GDNFR-γ2 polypeptide or the GDNFR-β, the GDNFR-γ1 or the GDNFR-γ2 polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or the nucleotide sequence of the GDNFR-β cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the GDNFR-β gene in human tissue, for instance, by Northern blot analysis. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:4 or SEQ ID NO:7 or the nucleotide sequence of the GDNFR-γ1 or the GDNFR-γ2 cDNA contained in the above-described deposited clones, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the GDNFR-γ1 or the GDNFR-γ2 gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNAs or the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:7) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments of about 50-1550 nt in length, and more preferrably at fragments least about 600 nt in length are also useful according to the present invention as are fragments

corresponding to most, if not all, of the nucleotide sequence of the deposited cDNAs or as shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:7). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNAs or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:7).

Preferred nucleic acid fragments of the present invention include nucleotides 1-280, 1-460, and 840-940 of Figure 1 (SEQ ID NO:1); nucleotides 1 - 1,111, 1-250, 250-500, 500-750, 750-1,111 of Figure 4 (SEQ ID NO:4); and nucleotides 1 - 1,111, 1-250, 250-500, 500-750, 750-1,111 of Figure 7 (SEQ ID NO:7) .

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the GDNFR-β receptor extracellular domain (predicted to constitute amino acid residues from about 22 to about 448 in Figure 1 (or amino acid residues from about 1 to about 427 in SEQ ID NO:2)); a polypeptide comprising the GDNFR-β receptor transmembrane domain (predicted to constitute amino acid residues from about 449 to about 464 in Figure 1 (or amino acid residues from about 428 to about 443 in SEQ ID NO:2)); and a polypeptide comprising the GDNFR-β receptor extracellular domain with all or part of the transmembrane domain deleted. As above with the leader sequence, the amino acid residues constituting the GDNFR-β receptor extracellular and transmembrane domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding: a polypeptide comprising the GDNFR-γ1 receptor extracellular domain (predicted to constitute amino acid residues from about 32 to about 360 in Figure 4 (or amino acid residues from about 1 to about

329 in SEQ ID NO:5)); a polypeptide comprising the GDNFR-γ1 receptor transmembrane domain (predicted to constitute amino acid residues from about 361 to about 378 in Figure 4 (or amino acid residues from about 330 to about 347 in SEQ ID NO:5)); and a polypeptide comprising the GDNFR-γ1 receptor extracellular domain with all or part of the transmembrane domain deleted. As above with the leader sequence, the amino acid residues constituting the GDNFR-γ1 receptor extracellular and transmembrane domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding: a polypeptide comprising the GDNFR-γ2 receptor extracellular domain (predicted to constitute amino acid residues from about 32 to about 382 in Figure 7 (or amino acid residues from about 1 to about 351 in SEQ ID NO:7)); a polypeptide comprising the GDNFR-γ2 receptor transmembrane domain (predicted to constitute amino acid residues from about 383 to about 400 in Figure 7 (or amino acid residues from about 352 to about 369 in SEQ ID NO:7)); and a polypeptide comprising the GDNFR-γ2 receptor extracellular domain with all or part of the transmembrane domain deleted. As above with the leader sequence, the amino acid residues constituting the GDNFR-γ2 receptor extracellular and transmembrane domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the GDNFR-β receptor protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 2 to about 59 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 68 to about 85 in SEQ ID NO:2; a

polypeptide comprising amino acid residues from about 98 to about 116 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 128 to about 200 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 205 to about 270 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 289 to about 374 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 411 to about 428 in SEQ ID NO:2. The inventors have determined that the above polypeptide fragments are antigenic regions of the GDNFR-β receptor.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the mature GDNFR-γ1 receptor protein. Such nucleic acid fragments of the present invention also include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 2 to about 10 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 13 to about 26 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 33 to about 40 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 42 to about 56 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 59 to about 67 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 71 to about 77 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 90 to about 114 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 122 to about 129 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 139 to about 164 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 174 to about 180 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 187 to about 203 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 217 to about 235 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 250 to about 257 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 302 to about 307 in SEQ ID NO:5; and a polypeptide comprising amino acid residues from about 317 to about 325 in SEQ ID NO:5.

The inventors have determined that the above polypeptide fragments are antigenic regions of the GDNFR-γ1 receptor.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the GDNFR-γ2 receptor protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 1 to about 9 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 14 to about 27 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 34 to about 41 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 43 to about 57 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 60 to about 68 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 72 to about 78 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 91 to about 115 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 122 to about 130 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 140 to about 165 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 175 to about 181 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 189 to about 204 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 216 to about 222 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 224 to about 236 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 251 to about 259 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 285 to about 299 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 314 to about 320 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 325 to about 330 in SEQ ID NO:7; and a polypeptide comprising amino acid residues from about 340 to about 348 in SEQ ID NO:7. The inventors have determined that the above polypeptide fragments are antigenic regions of the GDNFR-γ2 receptor.

Methods for determining other such epitope-bearing portions of the GDNFR-β, GDNFR-γ1or GDNFR-γ2 protein are described in detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit Nos. 97883, 209051 or 209052. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNAs or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:6).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the GDNFR-β receptor cDNA shown in Figure 1 (SEQ ID NO:1); the 3′ terminal poly(A) tract of the GDNFR-γ1 receptor cDNA shown in Figure 4 (SEQ ID NO:4); or the 3′ terminal poly(A) tract of the GDNFR-γ2 receptor cDNA shown in Figure 7 (SEQ ID NO:6)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of

the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide may include, but are not limited to those encoding the amino acid sequence of the mature polypeptides, by themselves; the coding sequence for the mature polypeptides and additional sequences, such as those encoding the amino acid leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*: 767 (1984). As discussed below, other such fusion proteins include the GDNFR-β receptor fused to Fc at the N- or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives

of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length GDNFR-β polypeptide having the complete amino acid sequence in SEQ ID NO:2, including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature GDNFR-β receptor (full-length polypeptide with the leader removed) having the amino acid sequence at positions from about 1 to about 443 in SEQ ID NO:2; (d) a nucleotide sequence encoding the full-length GDNFR-β polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No.97883; (e) a nucleotide sequence encoding the mature GDNFR-β receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883; (f) a nucleotide sequence encoding the GDNFR-β receptor extracellular domain; (g) a nucleotide sequence encoding the GDNFR-β receptor transmembrane domain; (h) a

nucleotide sequence encoding the GDNFR-β receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h).

Embodiments of the invention also include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length GDNFR-γ1 polypeptide having the complete amino acid sequence in Figure 4 (SEQ ID NO:5), including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:5, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature GDNFR-γ1 receptor (full-length polypeptide with the leader removed) having the amino acid sequence at positions from about 1 to about 347 in SEQ ID NO:5; (d) a nucleotide sequence encoding the full-length GDNFR-γ 1 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 209051; (e) a nucleotide sequence encoding the mature GDNFR-γ1 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051; (f) a nucleotide sequence encoding the GDNFR-γ1 receptor extracellular domain; (g) a nucleotide sequence encoding the GDNFR-γ1 receptor transmembrane domain; (h) a nucleotide sequence encoding the GDNFR-γ1 receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h).

Embodiments of the invention also include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length GDNFR-γ2 polypeptide having the complete amino acid sequence in Figure 7 (SEQ ID NO:7), including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID

NO:7, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature GDNFR-γ2 receptor (full-length polypeptide with the leader removed) having the amino acid sequence at positions from about 1 to about 369 in SEQ ID NO:7; (d) a nucleotide sequence encoding the full-length GDNFR-γ2 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 209052; (e) a nucleotide sequence encoding the mature GDNFR-γ2 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No.209052; (f) a nucleotide sequence encoding the GDNFR-γ2 receptor extracellular domain; (g) a nucleotide sequence encoding the GDNFR-γ2 receptor transmembrane domain; (h) a nucleotide sequence encoding the GDNFR-γ2 receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1, Figure 4 or Figure 7 or to the nucleotides sequence of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:6) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity include, *inter alia,* (1) isolating the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor

gene, as described in Verma *et al., Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:6) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity. By "a polypeptide having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor of the invention, as measured in a particular biological assay. For example, GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity can be measured using competition binding experiments of labeled GDNF or GDNF-like proteins to cells stably expressing the candidate GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide as described in Treanor *et al., Nature 382*:80-83 (1996) or Jing *et al., Cell 85*: 1113-1124 (1996).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNAs or the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 4 (SEQ ID NO:4) or Figure 7 (SEQ ID NO:6) will encode a polypeptide "having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having GDNFR-β, GDNFR-γ1 or GDNFR-γ2 protein activity. This is because the skilled artisan is

fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.,* "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide

moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5-receptor has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson e*t al.*, *The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a

recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

GDNFR-β, GDNFR-γ1 and GDNFR-γ2 Polypeptides and Fragments

The invention further provides an isolated GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide having the amino acid sequence encoded by the deposited cDNAs, or the amino acid sequence in Figure 1 (SEQ ID NO:2), Figure 4 (SEQ ID NO:5) or Figure 7 (SEQ ID NO:7), or a peptide or polypeptide comprising a portion of the above polypeptides.

It will be recognized in the art that some amino acid sequences of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Thus, the invention further includes variations of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor which show substantial GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity or which include regions of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID NO:2), Figure 4 (SEQ ID NO:5) Figure 7 (SEQ ID NO:7), or that encoded by the deposited cDNAs, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino

acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol. 2*:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

Also intended as an "isolated polypeptdide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the antimicrobial peptide polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67:*31-40 (1988).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67:*31-40 (1988).

The polypeptides of the present invention also include the polypeptide encoded by the deposited or GDNFR-β cDNA including the leader, the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 1 (SEQ ID NO:2) including the leader, the polypeptide of Figure 1 (SEQ ID NO:2) minus the leader, the extracellular domain, the transmembrane domain, a polypeptide comprising amino acids about -21 to about 443 in SEQ ID NO:2, and a polypeptide comprising amino acids about -20 to about 443 in SEQ ID NO:2, as well as polypeptides which are at least 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

The polypeptides of the present invention also include the polypeptide encoded by the deposited GDNFR-γ1 cDNA including the leader, the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 4 (SEQ ID NO:5) including the leader, the polypeptide of Figure 4 (SEQ ID NO:5) minus the leader, the extracellular domain, the transmembrane domain, a polypeptide comprising amino acids about -31 to about 347 in SEQ ID NO:5, and a polypeptide comprising amino acids about -30 to about 347 in SEQ ID NO:5, as well as polypeptides which are at least 95%

identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

The polypeptides of the present invention further include the polypeptide encoded by the deposited GDNFR-γ2 cDNA including the leader, the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 7 (SEQ ID NO:7) including the leader, the polypeptide of Figure 7 (SEQ ID NO:7) minus the leader, the extracellular domain, the transmembrane domain, a polypeptide comprising amino acids about -31 to about 369 in SEQ ID NO:7, and a polypeptide comprising amino acids about -30 to about 369 in SEQ ID NO:7, as well as polypeptides which are at least 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a GDNFR-β, GDNFR-γ1, or GDNFR-γ2 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figure 1 (SEQ ID NO:2), Figure 4 (SEQ ID NO:5) or Figure 7 (SEQ ID NO:7) or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting GDNFR-β, GDNFR-γ1, or GDNFR-γ2 expression as described below or as agonists and antagonists capable of enhancing or inhibiting GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor binding proteins which are also candidate agonist and antagonist according to the present invention. The yeast two hybrid system is described in Fields and Song, *Nature 340:*245-246 (1989).

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the

immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate GDNFR-β receptor-specific antibodies include: a polypeptide comprising amino acid residues from about 2 to about 59 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 68 to about 85 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 98 to about 116 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 128 to about 200 in SEQ ID NO:2; a polypeptide comprising amino acid residues from

about 205 to about 270 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 289 to about 374 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 411 to about 428 in SEQ ID NO:2. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the GDNFR-β receptor protein.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate mature GDNFR-γ1 receptor-specific antibodies include: Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the mature GDNFR-γ1 receptor protein. Such nucleic acid fragments of the present invention also include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 2 to about 10 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 13 to about 26 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 33 to about 40 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 42 to about 56 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 59 to about 67 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 71 to about 77 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 90 to about 114 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 122 to about 129 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 139 to about 164 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 174 to about 180 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 187 to about 203 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 217 to about 235 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 250 to about 257 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 302 to about 307 in SEQ ID NO:5; and a polypeptide comprising amino acid residues from about 317 to about 325 in SEQ ID NO:5. The inventors have determined that the above polypeptide fragments are antigenic regions of the GDNFR-γ1 receptor.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate GDNFR-γ2 receptor-specific antibodies include:a polypeptide comprising amino acid residues from about 1 to about 9 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 14 to about 27 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 34 to about 41 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 43 to about 57 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 60 to about 68 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 72 to about 78 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 91 to about 115 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 122 to about 130 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 140 to about 165 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 175 to about 181 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 189 to about 204 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 216 to about 222 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 224 to about 236 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 251 to about 259 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 285 to about 299 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 314 to about 320 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 325 to about 330 in SEQ ID NO:7; and a polypeptide comprising amino acid residues from about 340 to about 348 in SEQ ID NO:7. The inventors have determined that the above polypeptide fragments are antigenic regions of the GDNFR-γ2 receptor.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA 82*:5131-5135. This "Simultaneous Multiple Peptide Synthesis

(SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate, GDNFR-β, GDNFR-γ1, or GDNFR-γ2 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo.* This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al., Nature 331*:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein or protein fragment alone (Fountoulakis *et al., J. Biochem 270*:3958-3964 (1995)).

Diagnosis and Prognosis

It is believed that certain tissues in mammals with certain diseases and disorders express significantly decreased levels of the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor and mRNA encoding the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor when compared to a corresponding "standard" mammal, i.e., a mammal of the same species not having the disorder. Further, it is believed that enhanced levels of the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with cancer when compared to sera from mammals of the same species not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of diseases and disorders including Parkinson's disease, thyroid tumor, kidney failure and gut dysfunction, for example, which involves assaying the expression level of the gene encoding the GDNFR-β, GDNFR-γ1, or

GDNFR-γ2 receptor in mammalian cells or body fluid and comparing the gene expression level with a standard GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor gene expression level, whereby an decrease in the gene expression level over the standard is indicative of certain disorders.

Where a diagnosis of a disorder has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting decreased GDNFR-β, GDNFR-γ1, or GDNFR-γ2 gene expression will experience a worse clinical outcome relative to patients expressing the gene at a higher level.

By "assaying the expression level of the gene encoding the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein" is intended qualitatively or quantitatively measuring or estimating the level of the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein or the level of the mRNA encoding the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein level or mRNA level in a second biological sample).

Preferably, the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the cancer. As will be appreciated in the art, once a standard GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein or mRNA. Biological samples include mammalian body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein, and ovarian, prostate, heart, placenta, pancreas liver, spleen, lung, breast and umbilical tissue.

The present invention is useful for detecting disorders in mammals. In particular the invention is useful during diagnosis of the of following types of diseases and disorders in mammals: Parkinson's disease, schizophrenia, insomnia, tardive dyskenisia, hypertension, pituitary adenomas, hyperprolactinemia, thyroid tumor, renal disorders, kidney failure and gut dysfunction. Mutations that affect GDNFR-γ1 or GDNFR-γ2 sequence and/or expression levels of GDNFR-γ1 or GDNFR-γ2 could be diagnostic for patients with neurodegenerative diseases, particularly of the spinal motor neurons. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Total cellular RNA can be isolated from a biological sample using the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem. 162:*156-159 (1987). Levels of mRNA encoding the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor are then assayed using any appropriate method. These include Northern blot analysis (Harada *et al., Cell 63:*303-312 (1990)), S1 nuclease mapping (Fujita *et al., Cell 49:*357- 367 (1987)), the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR) (Fujita *et al., Cell 49:*357- 367 (1987)), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein levels in a biological sample can occur using antibody-based techniques. For example, GDNFR-β, GDNFR-γ1, or GDNFR-γ2 protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105:*3087-3096 (1987)). Other antibody-based methods useful for detecting GDNFR-β, GDNFR-γ1, or GDNFR-γ2 receptor gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable labels are known in the art and include enzyme labels, such as glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur

(^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Therapeutics

Studies that have analyzed the effects of defective GDNF expression in mice, have identified such disease states as Parkinson's disease, schizophrenia, insomnia, tardive dyskenisia, hypertension, pituitary adenomas, hyperprolactinemia, thyroid tumor, renal disorders, kidney failure and gut dysfunction as possibly being associated with the lack of a functional GDNF pathway. (Jing *et al., Cell 85*: 1113-1124 (1996); Trupp et al., Nature 381:785-789 (1996); Angrist *et al. Nat. Genet 14*:341-344 (1996); Vega *et al., Proc. Natl. Acad. Sci. 93*:10657-10661 (1996)). Defective GDNFR-β, GDNFR-γ1, or GDNFR-γ2 could cause or contribute to a dysfunctional GDNF pathway associated with these diseases and disorders. Thus, the GDNFR-β, GDNFR-γ1, or GDNFR-γ2 polypeptides of the present invention, as well as agonists of GDNFR-β, GDNFR-γ1, or GDNFR-γ2, could prove pharmacologically valuable factors for treating the above mentioned disease states.

It has been reported in the literature that spinal cord motor neurons are one of the major targets of GDNF action in vivo (Jing et al. 1996. Cell. 85, 1113-1124; Li et al. 1995. PNAS 92, 9771-9775; Oppenheim et al. 1995. Nature. 373, 344-346). GDNF can promote the survival of developing motoneurons as well as the survival of adult spinal motoneurons after injury in vivo. The predominant expression of GDNFR-γ1 or GDNFR-γ2 in spinal cord and its homology to GDNFR-α strongly suggest that GDNFR-γ1 or GDNFR-γ2 may directly mediate the protective effects of GDNF on spinal cord motor neurons. This suggests that patients with neurodegenerative diseases, such as degeneration of motor neurons as in amyotrophic lateral sclerosis (ALS) and of dopamine neurons in Parkinson's disease, may have defects in GDNFR-γ1 or GDNFR-γ2 that interfere with the ability to bind GDNF and inhibit neuron survival and

protection. Mutations that affect GDNFR-γ1 or GDNFR-γ2 sequence and/or expression levels of GDNFR-γ1 or GDNFR-γ2 could be diagnostic for patients with neurodegenerative diseases, particularly of the spinal motor neurons. In addition, soluble forms of GDNFR-γ1 or GDNFR-γ2 could be administered to such patients and could prove therapeutic. By analogy to GDNFR-α (Jing et al. 1996. Cell. 85, 1113-1124), soluble GDNFR-γ1 or GDNFR-γ2 should effectively bind to GDNF and the resulting GDNF-GDNFR-γ complex may then bind to Ret receptor and thereby transduce signals for neuron protection and/or survival.

The expression of GDNFR-γ1 or GDNFR-γ2 in a variety of fetal tissues, particularly fetal heart, skin, and liver, suggest that GDNFR-γ1 or GDNFR-γ2 may also play a role in the proliferation, differentiation, and/or survival of cells within these sites (e.g. cardiomyocytes; epithelium; hepatocytes). Alternatively, it may be involved in the establishment, maintenance, and/or differentiation of neurons within these regions of the embryo. The expression of GDNFR-γ1 or GDNFR-γ2 in fetal liver also suggests that it may play a role in hematopoiesis, as the fetal liver is a major site of hematopoiesis during the embryonic development. Possibly, GDNFR-γ1 or GDNFR-γ2 is involved in the proliferation or survival of early hematopoietic progenitor cells, or in the commitment or differentiation of hematopoietic cell lineages. Soluble GDNFR-γ1 or GDNFR-γ2 may be a useful therapeutic in effecting the regeneration of cardiomyocytes, epithelium, and hepatocytes in the adult organism. Likewise, soluble GDNFR-γ1 or GDNFR-γ2 may be useful in expanding the numbers of hematopoietic progenitors either *ex vivo* or *in vivo*.

Agonists and Antagonists of the GDNFR-β or GDNFR-γ

The present invention also provides a screening method for identifying compounds capable of enhancing or inhibiting a cellular response induced by the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor, which involves contacting cells which express the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor with the

candidate compound, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made in absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

In another aspect, a screening assay for agonists and antagonists is provided which involves determining the effect a candidate compound has on GDNF binding to the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor. In particular, the method involves contacting the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor with a GDNF (or GDNF-like) polypeptide and a candidate compound and determining whether GDNF polypeptide binding to the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor is increased or decreased due to the presence of the candidate compound.

By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known competition binding assays, including those described in more detail below.

One such screening procedure involves the use of melanophores which are transfected to express the receptor of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both GDNF as a ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

Other screening techniques include the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science 246*:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing in cells a construct wherein the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

Further screening assays for agonist and antagonist of the present invention are described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992).

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to GDNF. The method involves contacting cells which express the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide with a candidate compound and GDNF ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or GDNF (e.g., determining or estimating an increase or decrease in T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide can be contacted with either an endogenous or exogenously administered GDNF.

Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, GDNF peptide fragments, or other known compounds that behave as GDNF agonist. Preferred agonist include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and β-amyloid peptide. (*Science 267*:1457-1458 (1995)). Further preferred agonist include polyclonal and monoclonal antibodies raised against the GDNF-β, GDNFR-γ1 or GDNFR-γ2 polypeptide, or a fragment thereof.

Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, the CD40 ligand, neutral amino

acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *ElB*, Baculovirus *p35* and *IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1, LMP-1,* African swine fever virus *LMW5-HL,* and Herpesvirus yl 34.5), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and α-Hexachlorocyclohexane).

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem. 56*:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al., Nucleic Acids Research 6*:3073 (1979); Cooney *et al., Science 241*:456 (1988); and Dervan *et al., Science 251*:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptor.

Further antagonist according to the present invention include soluble forms of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 fragments that include the ligand binding domain from the extracellular region of the full length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize GDNFR-β, GDNFR-γ1 or GDNFR-γ2 mediated signaling by competing with the cell surface GDNFR-β, GDNFR-γ1 or GDNFR-γ2 for

binding to GDNF. These are preferably expressed as dimers or trimers, since these have been shown to be superior to monomeric forms of soluble receptor as antagonists, e.g., IgGFc-GDNFR-β or IgGFc-GDNFR-γ receptor family fusions.

Modes of administration

It will be appreciated that conditions caused by a decrease in the standard or normal level of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity in an individual, can be treated by administration of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 protein. Thus, the invention further provides a method of treating an individual in need of an increased level of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an isolated GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide of the invention, effective to increase the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity level in such an individual.

The invention also relates to a method of treating an individual in need of an increased level of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an agonist for GDNFR-β, GDNFR-γ1 or GDNFR-γ2. The invention further relates to a method of treating an individual in need of a decreased level of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor activity comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an antagonist for GDNFR-β, GDNFR-γ1 or GDNFR-γ2.

As a general proposition, the total pharmaceutically effective amount of GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide or its agonists or antagonists administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day,

and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Pharmaceutical compositions containing the GDNFR-β, GDNFR-γ1 or GDNFR-γ2 polypeptide(s)of the invention or its agonists or antagonsits may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Chromosome Assays

The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a GDNFR-β, GDNFR-γ1 or GDNFR-γ2 receptor gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose. In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer

analysis of the 3′ untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al., Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Expression and Purification of GDNFR-β in E. coli

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311).

pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra,* and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

The DNA sequence encoding the desired portion of the GDNFR-β protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the GDNFR-β protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the mature protein, the 5' primer has the sequence 5' CGC <u>CCATGG</u>CCAGCCCTTCCTCCCTG 3' (SEQ ID NO: 23) containing the underlined NcoI restriction site followed by 17 nucleotides complementary to the amino terminal coding sequence of the mature GDNFR-β sequence in Figure 1. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the mature form. The 3' primer has the sequence 5' CGC <u>AAG CTT</u> TTA CGG TCT GGC TCT GCT G 3' (SEQ ID NO: 24) containing the underlined HindIII restriction site followed by 16 nucleotides complementary to the 3' end of the non-coding sequence in the GDNFR-β DNA sequence in Figure 1.

The amplified GDNFR-β DNA fragments and the vector pQE60 are digested with NcoI/HindIII and the digested DNAs are then ligated together. Insertion of the GDNFR-β DNA into the restricted pQE60 vector places the GDNFR-β protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook *et al., Molecular Cloning: a Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing GDNFR-β protein, is available commercially from QIAGEN, Inc., *supra.* Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the

GDNFR-β is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure GDNFR-β protein. The purified protein is stored at 4 °C or frozen at -80 °C.

Example 2: Cloning and Expression of GDNFR-β protein in a Baculovirus Expression System

In this illustrative example, the plasmid shuttle vector pA2 was used to insert the cloned DNA encoding the complete protein, including its naturally associated secretary signal (leader) sequence, into a baculovirus to express the mature GDNFR-β protein, using standard methods as described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures,* Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI and Asp7l8. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an

in-frame AUG as required. Such vectors are described, for instance, in Luckow *et al., Virology 170*:31-39.

The cDNA sequence encoding the full length GDNFR-β protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in Figure 1 (SEQ ID NO:2), was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' CGC GGA TCC GCC ATC ATG ATC TTG GCA AAC GTC 3' (SEQ ID NO:25) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 18 bases of the sequence of the complete GDNFR-β protein shown in Figure 1, beginning with the AUG initiation codon. The 3' primer has the sequence 5' CGC GGT ACC TTA CGG TCT GGC TCT GCT GG 3' (SEQ ID NO:26) containing the underlined, Asp718 restriction site followed by 17 nucleotides complementary to nucleotides 1364-1380 in Figure 1, resulting in expression of a soluble receptor polypeptide. Alternatively, for expression of the full length receptor, the following 3' primer can be used: 5' CGC GGT ACC GCA AGG TGT GTG TGT GTC 3' (SEQ ID NO: 27) containing the underlined, Asp718 restriction site followed by 18 nucleotides complementary to nucleotides 1508- 1525 in Figure 1.

The amplified fragment was isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then was digested with BamHI/Asp718 and again is purified on a 1% agarose gel. This fragment is designated herein "F1".

The plasmid was digested with the restriction enzymes BamHI/Asp718 and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA was designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 were ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1

Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human GDNFR-β gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer was from well within the vector so that only those bacterial colonies containing the GDNFR-β gene fragment will show amplification of the DNA. The sequence of the cloned fragment was confirmed by DNA sequencing. This plasmid was designated herein pBac GDNFR-β.

Five μg of the plasmid pBac GDNFR-β was co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al., Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987). 1 μg of BaculoGold™ virus DNA and 5 μg of the plasmid pBac GDNFR-β were mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium were added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture was added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate was rocked back and forth to mix the newly added solution. The plate was then incubated for 5 hours at 27°C. After 5 hours the transfection solution was removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum was added. The plate was put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant was collected and a plaque assay was performed, as described by Summers and Smith, *supra.* An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) was used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life

Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques were picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses was then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus was used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes were harvested and then they were stored at 4 °C. The recombinant virus was called V-GDNFR-β.

To verify the expression of the GDNFR-β gene, Sf9 cells were grown in Grace's medium supplemented with 10% heat inactivated FBS. The cells were infected with the recombinant baculovirus V-GDNFR-β at a multiplicity of infection ("MOI") of about 2. Six hours later the medium was removed and was replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). If radiolabeled proteins were desired, 42 hours later, 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S-cysteine (available from Amersham) were added. The cells were further incubated for 16 hours and then they were harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins were analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the mature protein and thus the cleavage point and length of the secretory signal peptide.

Example 3: Cloning and Expression of GDNFR-β in Mammalian Cells

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late

promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al., Biochem J. 227*:277-279 (1991); Bebbington *et al., Bio/Technology 10:*169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al., Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al., Cell 41*:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, p GDNFR-β HA, is made by cloning a cDNA encoding GDNFR-β into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al., Cell 37:*767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the GDNFR-β is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The GDNFR-β cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of GDNFR-β in *E. coli.* Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined BamHI site, a Kozak sequence, an AUG start codon and 18 codons of the 5' coding region of the complete GDNFR-β has the following sequence: 5' CGC<u>GGATCC</u>GCCATCATGATCTTGGCAAACGTC 3' (SEQ ID NO: 28). The 3' primer, containing the underlined XbaI site, a stop codon, and 18 bp of 3'

coding sequence complementary to 1364-1380 in Figure 1 and has the following sequence (at the 3' end): 5' CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGGGTACGGTCTG GCTCTGCTGG 3' (SEQ ID NO: 29).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI, XbaI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the GDNFR-β-encoding fragment.

For expression of recombinant GDNFR-β, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al., Molecular Cloning: a Laboratory Manual,* Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of GDNFR-β by the vector.

Expression of the GDNFR-β-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow *et al., Antibodies: A Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of GDNFR-β protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J Biol. Chem. 253*:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta, 1097*:107-143, Page, M. J. and Sydenham, M.A. 1991, *Biotechnology 9*:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, *et al., Molecular and Cellular Biology,* March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al., Cell 41*:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β-actin promoter,

the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the GDNFR-β in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes BamHI/Asp718 and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete GDNFR-β protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' CGC GGA TCC GCC ATC ATG ATC TTG GCA AAC GTC 3' (SEQ ID NO:28) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 18 bases of the sequence of the complete GDNFR-β protein shown in Figure 1, beginning with the AUG initiation codon. The 3' primer has the sequence 5' CGC GGT ACC TTA CGG TCT GGC TCT GCT GG 3' (SEQ ID NO:26) containing the underlined, Asp718 restriction site followed by 17 nucleotides complementary to nucleotides 1364-1380 in Figure 1, resulting in expression of a soluble receptor polypeptide. Alternatively, for expression of the full length receptor, the following 3' primer can be used: 5' CGC GGT ACC GCA AGG TGT GTG TGT GTC 3' (SEQ ID NO:27) containing the underlined, Asp718 restriction site followed by 18 nucleotides complementary to nucleotides 1508- 1525 in Figure 1.

The amplified fragment is digested with the endonucleases BamHI and Asp718 and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. 5 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofectin (Felgner *et al., supra).* The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 4: Tissue distribution of GDNFR-β mRNA expression

Northern blot analysis was carried out to examine GDNFR-β gene expression in human tissues, using methods described by, among others, Sambrook *et al.,* cited above. A cDNA probe containing the entire nucleotide sequence of the GDNFR-β protein (SEQ ID NO: 1) was labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to

manufacturer's instructions. After labeling, the probe was purified using a CHROMA SPIN- 100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for GDNFR-β mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and were examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots were mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

The results demonstrated that GDNFR-β is expressed in most tissues. However, kidney, fetal kidney, fetal spleen, fetal lung, placenta, spleen, temporal lobe and pituitary gland have the highest levels of GDNFR-β expression.

Example 5: Expression and Purification of GDNFR-γ1 in E. coli

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide.

The DNA sequence encoding the desired portion GDNFR-γ1 protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the GDNFR-1γ protein and to sequences in

the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the mature protein, the 5' primer has the sequence 5' GCA GCA GCC ATG GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG AAC 3' (SEQ ID NO:30) containing the underlined NcoI restriction site followed by 32 nucleotides complementary to the amino terminal coding sequence of the mature GDNFR-γ1 sequence in Figure 4. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete protein shorter or longer than the mature form. The 3' primer has the sequence 5' GCA GCA AGA TCT CCA TAG GCT CAG GAG CAG AAT CAA GGG AAG 3' (SEQ ID NO: 31) containing the underlined BglII restriction site followed by 30 nucleotides complementary to the 3' end of the coding sequence immediately before the stop codon in the GDNFR-γ1 DNA sequence in Figure 4, with the coding sequence aligned with the restriction site so as to maintain its reading frame with that of the six His codons in the pQE60 vector.

For expression of the soluble GDNFR-γ1 protein the following primers are used as indicated above for the mature GDNFR-γ1 protein. The 5' primer has the sequence 5' GCA GCA GCC ATG GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG AAC 3' (SEQ ID NO:30) containing the underlined NcoI restriction site followed by 32 nucleotides complementary to the amino terminal coding sequence of the mature GDNFR-γ1 sequence in Figure 4. The 3' primer has the sequence 5' GCA GCA AGA TCT CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 32) containing the underlined BglII restriction site followed by 30 nucleotides complementary to the 3' end of the coding sequence immediately before the putative transmembrane domain in the GDNFR-γ1 DNA sequence in Figure 4, with the coding sequence aligned with the

restriction site so as to maintain its reading frame with that of the six His codons in the pQE60 vector.

The amplified GDNFR-γ1 DNA fragment and the vector pQE60 are digested with NcoI/BglII and the digested DNAs are then ligated together. Insertion of the GDNFR-γ1 DNA into the restricted pQE60 vector places the GDNFR-γ1 protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook *et al., Molecular Cloning: a Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing GDNFR-γ1 protein, is available commercially from QIAGEN, Inc., *supra.* Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the

GDNFR-γ1 is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure GDNFR-γ1 protein. The purified protein is stored at 4°C or frozen at -80°C.

Example 6: Cloning and Expression of GDNFR-γ1 protein in a Baculovirus Expression System

In this illustrative example, the plasmid shuttle vector pA2 is used to insert the cloned DNA encoding the complete protein, including its naturally associated secretary signal (leader) sequence, into a baculovirus to express the mature GDNFR-γ1 protein, using standard methods as described in Summers *et al., A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures,* Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for

transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow *et al., Virology 170*:31-39.

The cDNA sequence encoding the full length GDNFR-γ1 protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in Figure 4 (SEQ ID NO:5), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG 3' (SEQ ID NO: 33) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 30 bases of the sequence of the complete GDNFR-γ1 protein shown in Figure 4, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GCA GCA <u>GGT ACC</u> CTA CCA TAG GCT CAG GAG CAG AAT CAA GGG AAG 3' (SEQ ID NO: 34) containing the underlined, Asp718 restriction site followed by 33 nucleotides complementary nucleotides from position 1172-1203 in Figure 4.

For the expression of the soluble form of the GDNFR-γ1 protein, the following primers can be used to insert the cDNA into the baculovirus expression vector as indicated above. The 5' primer has the sequence 5' GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG 3' (SEQ ID NO:33) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 30 bases of the sequence of the complete GDNFR-γ protein shown in Figure 4, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GCA GCA

GGT ACC CTA CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 35) containing the underlined Asp718 restriction site followed by 33 nucleotides complementary to the nucleotides from position 1118-1147 in Figure 4.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with Bam HI and Asp718 and again is purified on a 1% agarose gel. This fragment is designated herein "F1".

The plasmid is digested with the restriction enzymes Bam HI and Asp718 and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human GDNFR-γ1 gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing the GDNFR-γ1 gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac GDNFR-γ1.

Five μg of the plasmid pBacGDNFR-γ1 is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al., Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987). 1 μg of BaculoGold™ virus DNA and 5 μg of the plasmid GDNFR-γ1 are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus

90 μl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra.* An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-GDNFR-γ1.

To verify the expression of the GDNFR-γ1 at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). If radiolabeled proteins are desired, 42 hours later, 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus

of purified protein may be used to determine the amino terminal sequence of the mature protein and thus the cleavage point and length of the secretory signal peptide.

Example 7: Cloning and Expression of GDNFR-γ in Mammalian Cells

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al., Biochem J. 227*:277-279 (1991); Bebbington *et al.,*

*Bio/Technology 10:*169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al., Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al., Cell 41*:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 7(a): Cloning and Expression of GDNFR-γ1 in COS Cells

The expression plasmid, pGDNFR-γ1HA, is made by cloning a cDNA encoding GDNFR-γ1 into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al., Cell 37:*767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery

of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the GDNFR-γ1 is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The GDNFR-γ1 cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of GDNFR-γ1 in *E. coli.* Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined BamHI site, a Kozak sequence, an AUG start codon and 30 nucleotides of the 5' coding region of the complete GDNFR-γ1 has the following sequence: 5' GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG 3' (SEQ ID NO: 33). The 3' primer, containing the underlined XbaI site, a stop codon, and 30 bp of 3' coding sequence has the following sequence (at the 3' end): 5' GCA GCA <u>TCT AGA</u> TCA AGC GTA GTC TGG GAC GTC GTA TGG GTA CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 36).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI/XbaI Please let us know if you would like us to prepare a patent application for this invention. and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the GDNFR-γ1 encoding fragment.

For expression of recombinant GDNFR-γ1, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described,

for instance, in Sambrook *et al., Molecular Cloning: a Laboratory Manual,* Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of GDNFR-γ1 by the vector.

Expression of the GDNFR-γ1-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow *et al., Antibodies: A Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 7(b): Cloning and Expression of GDNFR-γ1 in CHO Cells

The vector pC4 is used for the expression of GDNFR-γ1 protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J Biol. Chem. 253*:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta, 1097*:107-143, Page, M. J. and Sydenham, M.A. 1991, *Biotechnology 9*:64-68). Cells grown in increasing concentrations of MTX develop resistance to the

drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, *et al., Molecular and Cellular Biology,* March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al., Cell 41*:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the GDNFR-γ in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes Bam HI and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete GDNFR-γ 1 protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG 3' (SEQ ID NO: 33) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 30 bases of the coding sequence of GDNFR-γ1 shown in Figure 4 (SEQ ID NO:4). The 3' primer has the sequence 5' GCA GCA <u>AGA TCT</u> CTA CCA TAG GCT CAG GAG CAG AAT CAA GGG AAG 3' (SEQ ID NO: 37) containing the underlined BglII restriction site followed by 33 nucleotides complementary to the nucleotides from position 1172-1204 of the GDNFR-γ1 gene shown in Figure 4 (SEQ ID NO:4).

To express the soluble form of the GDNFR-γ1 protein the following primers are used as indicated above for the mature GDNFR-γ1 protein. The 5' primer has the sequence 5' GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG 3' (SEQ ID NO: 33) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 30 bases of the coding sequence of GDNFR-γ shown in Figure 4 (SEQ ID NO:4). The 3' primer has the sequence 5' GCA GCA <u>AGA TCT</u> CTA CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 38) containing the underlined 33 restriction site followed by 33 nucleotides complementary to the C-terminal region from position 1118-1147 of the GDNFR-γ1 gene shown in Figure 4 (SEQ ID NO:4).

The amplified fragment is digested with the endonucleases Bam HI and BglII and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. 5 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofectin (Felgner *et al., supra).* The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 7(c): Cloning and Expression of GDNFR-γ2 in COS Cells

The expression plasmid, pGDNFR-γ2 HA, is made by cloning a cDNA encoding GDNFR-γ2 into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an

ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al., Cell 37:*767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the GDNFR-γ2 is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The GDNFR-γ2 cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of GDNFR-γ2 in *E. coli.* Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined BamHI site, a Kozak sequence, an AUG start codon and 30 nucleotides of the 5' coding region of the complete GDNFR-γ2 has the following sequence: 5' GCA GCA <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG 3' (SEQ ID NO: 39). The 3' primer, containing the underlined XbaI site, a stop codon, and 30 bp of 3' coding sequence has the following sequence (at the 3' end): 5' GCA GCA <u>TCT AGA</u> TCA AGC GTA GTC TGG GAC GTC GTA TGG GTA CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO:36).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI/XbaI Please let us know if you would like us to prepare a patent application for this invention. and then ligated. The ligation mixture is

transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the GDNFR-γ2 encoding fragment.

For expression of recombinant GDNFR-γ2 , COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al., Molecular Cloning: a Laboratory Manual,* Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of GDNFR-γ2 by the vector.

Expression of the GDNFR-γ2-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow *et al., Antibodies: A Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 7(d): Cloning and Expression of GDNFR-γ2 in CHO Cells

The vector pC4 is used for the expression of GDNFR-γ2 protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate

activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J Biol. Chem. 253*:1357-1370, Hamlin, J. L. and Ma, C. 1990, *Biochem. et Biophys. Acta, 1097*:107-143, Page, M. J. and Sydenham, M.A. 1991, *Biotechnology 9*:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, *et al., Molecular and Cellular Biology,* March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al., Cell 41*:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human β-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the GDNFR-γ in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA 89*: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well.

Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes Bam HI and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete GDNFR-γ2 protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GCA CGC GGA TCC GCC ACC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG 3' (SEQ ID NO: 40) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 30 bases of the coding sequence of GDNFR-γ shown in Figure 7 (SEQ ID NO:6). The 3' primer has the sequence 5' GCA GCA AGA TCT CTA CCA TAG GCT CAG GAG CAG AAT CAA GGG AAG 3' (SEQ ID NO: 37) containing the underlined BglII restriction site followed by 33 nucleotides complementary to the nucleotides from position 1238-1271 of the GDNFR-γ2 gene shown in Figure 7 (SEQ ID NO:6).

To express the soluble form of the GDNFR-γ2 protein the following primers are used as indicated above for the mature GDNFR-γ2 protein. The 5' primer has the sequence 5' GCA CGC GGA TCC GCC ACC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG 3' (SEQ ID NO: 40) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 30 bases of the coding sequence of GDNFR-γ2 shown in Figure 7 (SEQ ID NO:6). The 3' primer has the sequence 5' GCA GCA AGA TCT CTA CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 38) containing the underlined 33 restriction site followed by 33 nucleotides

complementary to the C-terminal region from position 1184-1271 of the GDNFR-γ2 gene shown in Figure 7 (SEQ ID NO:6).

The amplified fragment is digested with the endonucleases Bam HI and BglII and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. 5 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofectin (Felgner *et al., supra).* The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 8: Tissue distribution of GDNFR-γ1 mRNA expression

Northern blot analysis was carried out to examine GDNFR-γ1 gene expression in human tissues, using methods described by, among others, Sambrook *et al.,* cited above. A cDNA probe containing the entire nucleotide

sequence of the GDNFR-γ1 protein (SEQ ID NO: 4) was labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe was purified using a CHROMA SPIN- 100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for GDNFR-γ1 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

Northern analysis demonstrates that the GDNFR-γ1 gene is expressed predominantly in adult spinal cord, and to a lesser extent in whole brain, hippocampus, and caudate nucleus (among a variety of tissues examined to date).

Example 9: Expression and Purification of GDNFR-γ2 in E. coli

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide.

The DNA sequence encoding the desired portion GDNFR-γ2 protein lacking the hydrophobic leader sequence is amplified from the deposited cDNA

clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the GDNFR-γ2 protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

For cloning the mature protein, the 5' primer has the sequence 5' GCA GCA GCC ATG GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG AAC 3' (SEQ ID NO:30) containing the underlined NcoI restriction site followed by 32 nucleotides complementary to the amino terminal coding sequence of the mature GDNFR-γ2 sequence in Figure 7. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete protein shorter or longer than the mature form. The 3' primer has the sequence 5' GCA GCA AGA TCT CCA TAG GCT CAG GAG CAG AAT CAA GGG AAG 3' (SEQ ID NO: 31) containing the underlined BglII restriction site followed by 30 nucleotides complementary to the 3' end of the coding sequence immediately before the stop codon in the GDNFR-γ2 DNA sequence in Figure 7, with the coding sequence aligned with the restriction site so as to maintain its reading frame with that of the six His codons in the pQE60 vector.

For expression of the soluble GDNFR-γ2 protein the following primers are used as indicated above for the mature GDNFR-γ2 protein. The 5' primer has the sequence 5' GCA GCA GCC ATG GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG AAC 3' (SEQ ID NO:30) containing the underlined NcoI restriction site followed by 32 nucleotides complementary to the amino terminal coding sequence of the mature GDNFR-γ2 sequence in Figure 7. The 3' primer has the sequence 5' GCA GCA AGA TCT CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 32) containing the underlined BglII restriction site followed by 30 nucleotides complementary to the 3' end of the coding sequence immediately before the putative transmembrane domain in the

GDNFR-γ2 DNA sequence in Figure 7, with the coding sequence aligned with the restriction site so as to maintain its reading frame with that of the six His codons in the pQE60 vector.

The amplified GDNFR-γ2 DNA fragment and the vector pQE60 are digested with NcoI/BglII and the digested DNAs are then ligated together. Insertion of the GDNFR-γ2 DNA into the restricted pQE60 vector places the GDNFR-γ2 protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook *et al., Molecular Cloning: a Laboratory Manual, 2nd Ed.;* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing GDNFR-γ2 protein, is available commercially from QIAGEN, Inc., *supra.* Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the GDNFR-γ2 is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure GDNFR-γ2 protein. The purified protein is stored at 4°C or frozen at -80°C.

Example 10: Cloning and Expression of GDNFR-γ2 protein in a Baculovirus Expression System

In this illustrative example, the plasmid shuttle vector pA2 is used to insert the cloned DNA encoding the complete protein, including its naturally associated secretary signal (leader) sequence, into a baculovirus to express the mature GDNFR-γ2 protein, using standard methods as described in Summers *et al., A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures,* Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow *et al., Virology 170*:31-39.

The cDNA sequence encoding the full length GDNFR-γ2 protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in Figure 7 (SEQ ID NO:7), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG 3' (SEQ ID NO: 39) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 30 bases of the sequence of the complete GDNFR-γ2 protein shown in Figure 7, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GCA GCA <u>GGT ACC</u> CTA CCA TAG GCT CAG GAG CAG AAT CAA GGG AAG 3' (SEQ ID NO: 34) containing the underlined, Asp718 restriction site followed by 33 nucleotides complementary nucleotides from position 1238-1271 in Figure 7.

For the expression of the soluble form of the GDNFR-γ2 protein, the following primers can be used to insert the cDNA into the baculovirus expression vector as indicated above. The 5' primer has the sequence 5' GCA CGC <u>GGA TCC</u> GCC ATC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG 3' (SEQ ID NO:39) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 30 bases of the sequence of the complete GDNFR-γ2 protein shown in Figure 7, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GCA GCA <u>GGT ACC</u> CTA CCA GGG CTG TGG CCT CAC AGC AGG GTT TTC 3' (SEQ ID NO: 35)

containing the underlined Asp718 restriction site followed by 33 nucleotides complementary to the nucleotides from position 1184-1217 in Figure 7.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with Bam HI and Asp718 and again is purified on a 1% agarose gel. This fragment is designated herein "F1".

The plasmid is digested with the restriction enzymes Bam HI and Asp718 and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human GDNFR-γ2 gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing the GDNFR-γ2 gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac GDNFR-γ2.

Five μg of the plasmid pBacGDNFR-γ2 is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al., Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987). 1 μg of BaculoGold™ virus DNA and 5 μg of the plasmid GDNFR-γ2 are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells

(ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra.* An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-GDNFR-γ2.

To verify the expression of the GDNFR-γ2 at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). If radiolabeled proteins are desired, 42 hours later, 5 μCi of ^{35}S-methionine and 5 μCi ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the

mature protein and thus the cleavage point and length of the secretory signal peptide.

Example 11: Tissue distribution of GDNFR-γ 2 mRNA expression

Northern blot analysis was carried out to examine GDNFR-γ2 gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the GDNFR-γ2 protein (SEQ ID NO: 6) was labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe was purified using a CHROMA SPIN- 100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for GDNFR-γ2 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

Northern analysis demonstrates that the GDNFR-γ2 gene is expressed predominantly in adult spinal cord, and to a lesser extent in whole brain, hippocampus, and caudate nucleus (among a variety of tissues examined to date).

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: GDNF RECEPTORS

(iii) NUMBER OF SEQUENCES: 40

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(E) COUNTRY: US
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: HEREWITH
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/047,092
(B) FILING DATE: 20-MAY-1997

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1543 base pairs
(B) TYPE: nucleic acid

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        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA


    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 37..1428

    (ix) FEATURE:
        (A) NAME/KEY: mat_peptide
        (B) LOCATION: 100..1428

    (ix) FEATURE:
        (A) NAME/KEY: sig_peptide
        (B) LOCATION: 37..99


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGAGAAAGA CAAAAAAACG GTGGGATTTA TTTAAC ATG ATC TTG GCA AAC GTC         54
                                        Met Ile Leu Ala Asn Val
                                        -21 -20

TTC TGC CTC TTC TTC TTT CTA GAC GAG ACC CTC CGC TCT TTG GCC AGC        102
Phe Cys Leu Phe Phe Phe Leu Asp Glu Thr Leu Arg Ser Leu Ala Ser
-15                 -10                 -5                    1

CCT TCC TCC CTG CAG GGC CCC GAG CTC CAC GGC TGG CGC CCC CCA GTG        150
Pro Ser Ser Leu Gln Gly Pro Glu Leu His Gly Trp Arg Pro Pro Val
              5                  10                  15

GAC TGT GTC CGG GCC AAT GAG CTG TGT GCC GCC GAA TCC AAC TGC AGC        198
Asp Cys Val Arg Ala Asn Glu Leu Cys Ala Ala Glu Ser Asn Cys Ser
         20                  25                  30

TCT CGC TAC CGC ACT CTG CGG CAG TGC CTG GCA GGC CGC GAC CGC AAC        246
Ser Arg Tyr Arg Thr Leu Arg Gln Cys Leu Ala Gly Arg Asp Arg Asn
     35                  40                  45

ACC ATG CTG GCC AAC AAG GAG TGC CAG GCG GCC TTG GAG GTC TTG CAG        294
Thr Met Leu Ala Asn Lys Glu Cys Gln Ala Ala Leu Glu Val Leu Gln
 50                  55                  60                  65

GAG AGC CCG CTG TAC GAC TGC CGC TGC AAG CGG GGC ATG AAG AAG GAG        342
Glu Ser Pro Leu Tyr Asp Cys Arg Cys Lys Arg Gly Met Lys Lys Glu
                 70                  75                  80

CTG CAG TGT CTG CAG ATC TAC TGG AGC ATC CAC CTG GGG CTG ACC GAG        390
Leu Gln Cys Leu Gln Ile Tyr Trp Ser Ile His Leu Gly Leu Thr Glu
             85                  90                  95

GGT GAG GAG TTC TAC GAA GCC TCC CCC TAT GAG CCG GTG ACC TCC CGC        438
Gly Glu Glu Phe Tyr Glu Ala Ser Pro Tyr Glu Pro Val Thr Ser Arg
        100                 105                 110

CTC TCG GAC ATC TTC AGG CTT GCT TCA ATC TTC TCA GGG ACA GGG GCA        486
Leu Ser Asp Ile Phe Arg Leu Ala Ser Ile Phe Ser Gly Thr Gly Ala
    115                 120                 125

GAC CCG GTG GTC AGC GCC AAG AGC AAC CAT TGC CTG GAT GCT GCC AAG        534
```

```
Asp Pro Val Val Ser Ala Lys Ser Asn His Cys Leu Asp Ala Ala Lys
130                 135                 140                 145

GCC TGC AAC CTG AAT GAC AAC TGC AAG AAG CTG CGC TCC TCC TAC ATC      582
Ala Cys Asn Leu Asn Asp Asn Cys Lys Lys Leu Arg Ser Ser Tyr Ile
                150                 155                 160

TCC ATC TGC AAC CGC GAG ATC TCG CCC ACC GAG CGC TGC AAC CGC CGC      630
Ser Ile Cys Asn Arg Glu Ile Ser Pro Thr Glu Arg Cys Asn Arg Arg
            165                 170                 175

AAG TGC CAC AAG GCC CTG CGC CAG TTC TTC GAC CGG GTG CCC AGC GAG      678
Lys Cys His Lys Ala Leu Arg Gln Phe Phe Asp Arg Val Pro Ser Glu
        180                 185                 190

TAC ACC TAC CGC ATG CTC TTC TGC TCC TGC CAA GAC CAG GCG TGC GCT      726
Tyr Thr Tyr Arg Met Leu Phe Cys Ser Cys Gln Asp Gln Ala Cys Ala
    195                 200                 205

GAG CGC CGC CGG CAA ACC ATC CTG CCC AGC TGC TCC TAT GAG GAC AAG      774
Glu Arg Arg Arg Gln Thr Ile Leu Pro Ser Cys Ser Tyr Glu Asp Lys
210                 215                 220                 225

GAG AAG CCC AAC TGC CTG GAC CTG CGT GGC GTG TGC CGG ACT GAC CAC      822
Glu Lys Pro Asn Cys Leu Asp Leu Arg Gly Val Cys Arg Thr Asp His
                230                 235                 240

CTG TGT CGG TCC CGG CTG GCC GAC TTC CAT GCC AAT TGT CGA GCC TCC      870
Leu Cys Arg Ser Arg Leu Ala Asp Phe His Ala Asn Cys Arg Ala Ser
            245                 250                 255

TAC CAG ACG GTC ACC AGC TGC CCT GCG GAC AAT TAC CAG GCG TGT CTG      918
Tyr Gln Thr Val Thr Ser Cys Pro Ala Asp Asn Tyr Gln Ala Cys Leu
        260                 265                 270

GGC TCT TAT GCT GGC ATG ATT GGG TTT GAC ATG ACA CCT AAC TAT GTG      966
Gly Ser Tyr Ala Gly Met Ile Gly Phe Asp Met Thr Pro Asn Tyr Val
    275                 280                 285

GAC TCC AGC CCC ACT GGC ATC GTG GTG TCC CCC TGG TGC AGC TGT CGT     1014
Asp Ser Ser Pro Thr Gly Ile Val Val Ser Pro Trp Cys Ser Cys Arg
290                 295                 300                 305

GGC AGC GGG AAC ATG GAG GAG GAG TGT GAG AAG TTC CTC AGG GAC TTC     1062
Gly Ser Gly Asn Met Glu Glu Glu Cys Glu Lys Phe Leu Arg Asp Phe
                310                 315                 320

ACC GAG AAC CCA TGC CTC CGG AAC GCC ATC CAG GCC TTT GGC AAC GGC     1110
Thr Glu Asn Pro Cys Leu Arg Asn Ala Ile Gln Ala Phe Gly Asn Gly
            325                 330                 335

ACG GAT GTG AAC GTG TCC CCA AAA GGC CCC TCG TTC CAG GCC ACC CAG     1158
Thr Asp Val Asn Val Ser Pro Lys Gly Pro Ser Phe Gln Ala Thr Gln
        340                 345                 350

GCC CCT CGG GTG GAG AAG ACG CCT TCT TTG CCA GAT GAC CTC AGT GAC     1206
Ala Pro Arg Val Glu Lys Thr Pro Ser Leu Pro Asp Asp Leu Ser Asp
    355                 360                 365

AGT ACC AGC TTG GGG ACC AGT GTC ATC ACC ACC TGC ACG TCT GTC CAG     1254
Ser Thr Ser Leu Gly Thr Ser Val Ile Thr Thr Cys Thr Ser Val Gln
370                 375                 380                 385
```

GAG CAG GGG CTG AAG GCC AAC AAC TCC AAA GAG TTA AGC ATG TGC TTC 1302
Glu Gln Gly Leu Lys Ala Asn Asn Ser Lys Glu Leu Ser Met Cys Phe
390 395 400

ACA GAG CTC ACG ACA AAT ATC ATC CCA GGG AGT AAC AAG GTG ATC AAA 1350
Thr Glu Leu Thr Thr Asn Ile Ile Pro Gly Ser Asn Lys Val Ile Lys
405 410 415

CCT AAC TCA GGC CCC AGC AGA GCC AGA CCG TCG GCT GCC TTG ACC GTG 1398
Pro Asn Ser Gly Pro Ser Arg Ala Arg Pro Ser Ala Ala Leu Thr Val
420 425 430

CTG TCT GTC CTG ATG CTG AAA CTG GCC TTG TAGGCTGTGG GAACCGAGTC 1448
Leu Ser Val Leu Met Leu Lys Leu Ala Leu
435 440

AGAAGATTTT TGAAATACGC AGACAAGAAC AGCCGCCTGA CGAAATGGAA ACACACACAG 1508

ACACACACAC ACCTTGCAAA AAAAAAAAAA AAAAA 1543

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 464 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ile Leu Ala Asn Val Phe Cys Leu Phe Phe Phe Leu Asp Glu Thr
-21 -20 -15 -10

Leu Arg Ser Leu Ala Ser Pro Ser Ser Leu Gln Gly Pro Glu Leu His
-5 1 5 10

Gly Trp Arg Pro Pro Val Asp Cys Val Arg Ala Asn Glu Leu Cys Ala
15 20 25

Ala Glu Ser Asn Cys Ser Ser Arg Tyr Arg Thr Leu Arg Gln Cys Leu
30 35 40

Ala Gly Arg Asp Arg Asn Thr Met Leu Ala Asn Lys Glu Cys Gln Ala
45 50 55

Ala Leu Glu Val Leu Gln Glu Ser Pro Leu Tyr Asp Cys Arg Cys Lys
60 65 70 75

Arg Gly Met Lys Lys Glu Leu Gln Cys Leu Gln Ile Tyr Trp Ser Ile
80 85 90

His Leu Gly Leu Thr Glu Gly Glu Glu Phe Tyr Glu Ala Ser Pro Tyr
95 100 105

Glu Pro Val Thr Ser Arg Leu Ser Asp Ile Phe Arg Leu Ala Ser Ile
110 115 120

Phe Ser Gly Thr Gly Ala Asp Pro Val Val Ser Ala Lys Ser Asn His
125 130 135

Cys Leu Asp Ala Ala Lys Ala Cys Asn Leu Asn Asp Asn Cys Lys Lys
140 145 150 155

Leu Arg Ser Ser Tyr Ile Ser Ile Cys Asn Arg Glu Ile Ser Pro Thr
160 165 170

Glu Arg Cys Asn Arg Arg Lys Cys His Lys Ala Leu Arg Gln Phe Phe
175 180 185

Asp Arg Val Pro Ser Glu Tyr Thr Tyr Arg Met Leu Phe Cys Ser Cys
190 195 200

Gln Asp Gln Ala Cys Ala Glu Arg Arg Arg Gln Thr Ile Leu Pro Ser
205 210 215

Cys Ser Tyr Glu Asp Lys Glu Lys Pro Asn Cys Leu Asp Leu Arg Gly
220 225 230 235

Val Cys Arg Thr Asp His Leu Cys Arg Ser Arg Leu Ala Asp Phe His
240 245 250

Ala Asn Cys Arg Ala Ser Tyr Gln Thr Val Thr Ser Cys Pro Ala Asp
255 260 265

Asn Tyr Gln Ala Cys Leu Gly Ser Tyr Ala Gly Met Ile Gly Phe Asp
270 275 280

Met Thr Pro Asn Tyr Val Asp Ser Ser Pro Thr Gly Ile Val Val Ser
285 290 295

Pro Trp Cys Ser Cys Arg Gly Ser Gly Asn Met Glu Glu Glu Cys Glu
300 305 310 315

Lys Phe Leu Arg Asp Phe Thr Glu Asn Pro Cys Leu Arg Asn Ala Ile
320 325 330

Gln Ala Phe Gly Asn Gly Thr Asp Val Asn Val Ser Pro Lys Gly Pro
335 340 345

Ser Phe Gln Ala Thr Gln Ala Pro Arg Val Glu Lys Thr Pro Ser Leu
350 355 360

Pro Asp Asp Leu Ser Asp Ser Thr Ser Leu Gly Thr Ser Val Ile Thr
365 370 375

Thr Cys Thr Ser Val Gln Glu Gln Gly Leu Lys Ala Asn Asn Ser Lys
380 385 390 395

Glu Leu Ser Met Cys Phe Thr Glu Leu Thr Thr Asn Ile Ile Pro Gly
400 405 410

Ser Asn Lys Val Ile Lys Pro Asn Ser Gly Pro Ser Arg Ala Arg Pro
415 420 425

Ser Ala Ala Leu Thr Val Leu Ser Val Leu Met Leu Lys Leu Ala Leu
430 435 440

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Phe Leu Ala Thr Leu Tyr Phe Ala Leu Pro Leu Leu Asp Leu Leu
1               5                   10                  15

Met Ser Ala Glu Val Ser Gly Gly Asp Arg Leu Asp Cys Val Lys Ala
            20                  25                  30

Ser Asp Gln Cys Leu Lys Glu Gln Ser Cys Ser Thr Lys Tyr Arg Thr
        35                  40                  45

Leu Arg Gln Cys Val Ala Gly Lys Glu Thr Asn Phe Ser Leu Thr Ser
    50                  55                  60

Gly Leu Glu Ala Lys Asp Glu Cys Arg Ser Ala Met Glu Ala Leu Lys
65                  70                  75                  80

Gln Lys Ser Leu Tyr Asn Cys Arg Cys Lys Arg Gly Met Lys Lys Glu
                85                  90                  95

Lys Asn Cys Leu Arg Ile Tyr Trp Ser Met Tyr Gln Ser Leu Gln Gly
            100                 105                 110

Asn Asp Leu Leu Glu Asp Ser Pro Tyr Glu Pro Val Asn Ser Arg Leu
        115                 120                 125

Ser Asp Ile Phe Arg Ala Val Pro Phe Ile Ser Asp Val Phe Gln Gln
    130                 135                 140

Val Glu His Ile Ser Lys Gly Asn Asn Cys Leu Asp Ala Ala Lys Ala
145                 150                 155                 160

Cys Asn Leu Asp Asp Thr Cys Lys Lys Tyr Arg Ser Ala Tyr Ile Thr
                165                 170                 175

Pro Cys Thr Thr Ser Met Ser Asn Glu Val Cys Asn Arg Arg Lys Cys
            180                 185                 190

His Lys Ala Leu Arg Gln Phe Phe Asp Lys Val Pro Ala Lys His Ser
        195                 200                 205

Tyr Gly Met Leu Phe Cys Ser Cys Arg Asp Ile Ala Cys Thr Glu Arg
    210                 215                 220

Arg Arg Gln Thr Ile Val Pro Val Cys Ser Tyr Glu Glu Arg Glu Arg
225                 230                 235                 240

Pro Asn Cys Leu Ser Leu Gln Asp Ser Cys Lys Thr Asn Tyr Ile Cys
                245                 250                 255

Arg Ser Arg Leu Ala Asp Phe Phe Thr Asn Cys Gln Pro Glu Ser Arg
            260                 265                 270
```

```
Ser Val Ser Asn Cys Leu Lys Glu Asn Tyr Ala Asp Cys Leu Leu Ala
        275                 280                 285

Tyr Ser Gly Leu Ile Gly Thr Val Met Thr Pro Asn Tyr Val Asp Ser
    290                 295                 300

Ser Ser Leu Ser Val Ala Pro Trp Cys Asp Cys Ser Asn Ser Gly Asn
305                 310                 315                     320

Asp Leu Glu Asp Cys Leu Lys Phe Leu Asn Phe Phe Lys Asp Asn Thr
                325                 330                 335

Cys Leu Lys Asn Ala Ile Gln Ala Phe Gly Asn Gly Ser Asp Val Thr
            340                 345                 350

Met Trp Gln Pro Ala Pro Pro Val Gln Thr Thr Thr Ala Thr Thr Thr
        355                 360                 365

Thr Ala Phe Arg Val Lys Asn Lys Pro Leu Gly Pro Ala Gly Ser Glu
    370                 375                 380

Asn Glu Ile Pro Thr His Val Leu Pro Pro Cys Ala Asn Leu Gln Ala
385                 390                 395                     400

Gln Lys Leu Lys Ser Asn Val Ser Gly Ser Thr His Leu Cys Leu Ser
                405                 410                 415

Asp Ser Asp Phe Gly Lys Asp Gly Leu Ala Gly Ala Ser Ser His Ile
            420                 425                 430

Thr Thr Lys Ser Met Ala Ala Pro Pro Ser Cys Ser Leu Ser Ser Leu
        435                 440                 445

Pro Val Leu Met Leu Thr
    450
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1809 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 68..1201

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 161..1201

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 68..160

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GAGCCCCGCT CTCAGAGCTC CAGGGGAGGA GCGAGGGGAG CGCGGAGCCC GGCGCCTACA      60

GCTCGCC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC        109
        Met Val Arg Pro Leu Asn Pro Arg Pro Leu Pro Pro Val Val
        -31 -30                 -25                 -20

CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC        157
Leu Met Leu Leu Leu Leu Leu Pro Pro Ser Pro Leu Pro Leu Ala Ala
        -15                 -10                  -5

GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG AAC AGC TGT CTC CAG        205
Gly Asp Pro Leu Pro Thr Glu Ser Arg Leu Met Asn Ser Cys Leu Gln
      1               5              10                      15

GCC AGG AGG AAG TGC CAG GCT GAT CCC ACC TGC AGT GCT GCC TAC CAC        253
Ala Arg Arg Lys Cys Gln Ala Asp Pro Thr Cys Ser Ala Ala Tyr His
                 20                  25                  30

CAC CTG GAT TCC TGC ACC TCT AGC ATA AGC ACC CCA CTG CCC TCA GAG        301
His Leu Asp Ser Cys Thr Ser Ser Ile Ser Thr Pro Leu Pro Ser Glu
             35                  40                  45

GAG CCT TCG GTC CCT GCT GAC TGC CTG GAG GCA GCA CAG CAA CTC AGG        349
Glu Pro Ser Val Pro Ala Asp Cys Leu Glu Ala Ala Gln Gln Leu Arg
         50                  55                  60

AAC AGC TCT CTG ATA GGC TGC ATG TGC CAC CGG CGC ATG AAG AAC CAG        397
Asn Ser Ser Leu Ile Gly Cys Met Cys His Arg Arg Met Lys Asn Gln
     65                  70                  75

GTT GCC TGC TTG GAC ATC TAT TGG ACC GTT CAC CGT GCC CGC AGC CTT        445
Val Ala Cys Leu Asp Ile Tyr Trp Thr Val His Arg Ala Arg Ser Leu
 80                  85                  90                  95

GGT AAC TAT GAG CTG GAT GTC TCC CCC TAT GAA GAC ACA GTG ACC AGC        493
Gly Asn Tyr Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser
                100                 105                 110

AAA CCC TGG AAA ATG AAT CTC AGC AAA CTG AAC ATG CTC AAA CCA GAC        541
Lys Pro Trp Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp
            115                 120                 125

TCA GAC CTC TGC CTC AAG TTT GCC ATG CTG TGT ACT CTC AAT GAC AAG        589
Ser Asp Leu Cys Leu Lys Phe Ala Met Leu Cys Thr Leu Asn Asp Lys
        130                 135                 140

TGT GAC CGG CTG CGC AAG GCC TAC GGG GAG GCG TGC TCC GGG CCC CAC        637
Cys Asp Arg Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Pro His
    145                 150                 155

TGC CAG CGC CAC GTC TGC CTC AGG CAG CTG CTC ACT TTC TTC GAG AAG        685
Cys Gln Arg His Val Cys Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys
160                 165                 170                 175

GCC GCC GAG CCC CAC GCG CAG GGC CTG CTA CTG TGC CCA TGT GCC CCC        733
Ala Ala Glu Pro His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro
                180                 185                 190

AAC GAC CGG GGC TGC GGG GAG CGC CGG CGC AAC ACC ATC GCC CCC AAC        781
Asn Asp Arg Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn
            195                 200                 205
```

TGC GCG CTG CCG CCT GTG GCC CCC AAC TGC CTG GAG CTG CGG CGC CTC 829
Cys Ala Leu Pro Pro Val Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu
210 215 220

TGC TTC TCC GAC CCG CTT TGC AGA TCA CGC CTG GTG GAT TTC CAG ACC 877
Cys Phe Ser Asp Pro Leu Cys Arg Ser Arg Leu Val Asp Phe Gln Thr
225 230 235

CAC TGC CAT CCC ATG GAC ATC CTA GGA ACT TGT GCA ACA GAG CAG TCC 925
His Cys His Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser
240 245 250 255

AGA TGT CTA CGA GCA TAC CTG GGG CTG ATT GGG ACT GCC ATG ACC CCC 973
Arg Cys Leu Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro
260 265 270

AAC TTT GTC AGC AAT GTC AAC ACC AGT GTT GCC TTA AGC TGC ACC TGC 1021
Asn Phe Val Ser Asn Val Asn Thr Ser Val Ala Leu Ser Cys Thr Cys
275 280 285

CTC ACG GAG GCC ATT GCA GCT AAG ATG CGT TTT CAC AGC CAA CTC TTC 1069
Leu Thr Glu Ala Ile Ala Ala Lys Met Arg Phe His Ser Gln Leu Phe
290 295 300

TCC CAG GAC TGG CCA CAC CCT ACC TTT GCT GTG ATG GCA CAC CAG AAT 1117
Ser Gln Asp Trp Pro His Pro Thr Phe Ala Val Met Ala His Gln Asn
305 310 315

GAA AAC CCT GCT GTG AGG CCA CAG CCC TGG GTG CCC TCT CTT TTC TCC 1165
Glu Asn Pro Ala Val Arg Pro Gln Pro Trp Val Pro Ser Leu Phe Ser
320 325 330 335

TGC ACG CTT CCC TTG ATT CTG CTC CTG AGC CTA TGG TAGCTGGACT 1211
Cys Thr Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp
340 345

TCCCCAGGGC CCTCTTCCCC TCCACCACAC CCAGGTGGAC TTGCAGCCCA CAAGGGGTGA 1271

GGAAAGGACA GCAGCAGGAA GGAGGTGCAG TGCGCAGATG AGGGCACAGG AGAAGCTAAG 1331

GGTTATGACC TCCAGATCCT TACTGGTCCA GTCCTCATTC CCTCCACCCC ATCTCCACTT 1391

CTGATTCATG CTGCCCCTCC TTGGTGGCCA CAATTTAGCC ATGTCATCTG GTGGTGACCA 1451

GCTCCACCAA GCCCCTTTCT GAGCCCTTCC TCTTGACTAC CAGGATCACC AGAATCTAAT 1511

AAGTTAGCCT TTCTCTATTG CATTCCAGAT TAGGGTTAGG GTAGGGAGGA CTGGGTGTTC 1571

TGAGGCAGCC TAGAAAGTCA TTCTCCTTTG TGAAGAAGGC TCCTGCCCCC TCGTCTCCTC 1631

CTCTGAGTGG AGGATGGAAA ACTACTGCCT GCACTGCCCT GTCCCCGGAT CCTGCCGAAC 1691

ATCTGGGCAT CAGGAGCTGG AGCCTGTGGG CCTTGCTTTA TTCCTATTAT TGTCCTAAAG 1751

TCTCTCTGGG CTCTTGGATC ATGATTAAAC CTTTGACTTA AAAAAAAAAA AAAAAAAA 1809

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 378 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Val Arg Pro Leu Asn Pro Arg Pro Leu Pro Pro Val Val Leu Met
-31 -30                 -25                 -20

Leu Leu Leu Leu Leu Pro Pro Ser Pro Leu Pro Leu Ala Ala Gly Asp
-15                 -10                  -5                   1

Pro Leu Pro Thr Glu Ser Arg Leu Met Asn Ser Cys Leu Gln Ala Arg
              5                  10                  15

Arg Lys Cys Gln Ala Asp Pro Thr Cys Ser Ala Ala Tyr His His Leu
         20                  25                  30

Asp Ser Cys Thr Ser Ser Ile Ser Thr Pro Leu Pro Ser Glu Glu Pro
     35                  40                  45

Ser Val Pro Ala Asp Cys Leu Glu Ala Ala Gln Gln Leu Arg Asn Ser
 50                  55                  60                  65

Ser Leu Ile Gly Cys Met Cys His Arg Arg Met Lys Asn Gln Val Ala
                 70                  75                  80

Cys Leu Asp Ile Tyr Trp Thr Val His Arg Ala Arg Ser Leu Gly Asn
             85                  90                  95

Tyr Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro
        100                 105                 110

Trp Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser Asp
    115                 120                 125

Leu Cys Leu Lys Phe Ala Met Leu Cys Thr Leu Asn Asp Lys Cys Asp
130                 135                 140                 145

Arg Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Pro His Cys Gln
                150                 155                 160

Arg His Val Cys Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys Ala Ala
            165                 170                 175

Glu Pro His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Asn Asp
        180                 185                 190

Arg Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn Cys Ala
    195                 200                 205

Leu Pro Pro Val Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu Cys Phe
210                 215                 220                 225

Ser Asp Pro Leu Cys Arg Ser Arg Leu Val Asp Phe Gln Thr His Cys
                230                 235                 240

His Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys
            245                 250                 255

Leu Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn Phe
        260                 265                 270
```

```
Val Ser Asn Val Asn Thr Ser Val Ala Leu Ser Cys Thr Cys Leu Thr
    275                 280                 285

Glu Ala Ile Ala Ala Lys Met Arg Phe His Ser Gln Leu Phe Ser Gln
290                 295                 300                 305

Asp Trp Pro His Pro Thr Phe Ala Val Met Ala His Gln Asn Glu Asn
                310                 315                 320

Pro Ala Val Arg Pro Gln Pro Trp Val Pro Ser Leu Phe Ser Cys Thr
            325                 330                 335

Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp
        340                 345
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1878 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 68..1267

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 161..1267

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 68..160

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GAGCCCCGCT CTCAGAGCTC CAGGGGAGGA GCGAGGGGAG CGCGGAGCCC GGCGCCTACA      60

GCTCGCC ATG GTG CGC CCC CTG AAC CCG CGA CCG CTG CCG CCC GTA GTC       109
        Met Val Arg Pro Leu Asn Pro Arg Pro Leu Pro Pro Val Val
        -31 -30                 -25                 -20

CTG ATG TTG CTG CTG CTG CTG CCG CCG TCG CCG CTG CCT CTC GCA GCC       157
Leu Met Leu Leu Leu Leu Leu Pro Pro Ser Pro Leu Pro Leu Ala Ala
        -15                 -10                 -5

GGA GAC CCC CTT CCC ACA GAA AGC CGA CTC ATG AAC AGC TGT CTC CAG       205
Gly Asp Pro Leu Pro Thr Glu Ser Arg Leu Met Asn Ser Cys Leu Gln
      1               5                  10                  15

GCC AGG AGG AAG TGC CAG GCT GAT CCC ACC TGC AGT GCT GCC TAC CAC       253
Ala Arg Arg Lys Cys Gln Ala Asp Pro Thr Cys Ser Ala Ala Tyr His
                 20                  25                  30

CAC CTG GAT TCC TGC ACC TCT AGC ATA AGC ACC CCA CTG CCC TCA GAG       301
His Leu Asp Ser Cys Thr Ser Ser Ile Ser Thr Pro Leu Pro Ser Glu
            35                  40                  45
```

```
GAG CCT TCG GTC CCT GCT GAC TGC CTG GAG GCA GCA CAG CAA CTC AGG        349
Glu Pro Ser Val Pro Ala Asp Cys Leu Glu Ala Ala Gln Gln Leu Arg
         50                  55                  60

AAC AGC TCT CTG ATA GGC TGC ATG TGC CAC CGG CGC ATG AAG AAC CAG        397
Asn Ser Ser Leu Ile Gly Cys Met Cys His Arg Arg Met Lys Asn Gln
     65                  70                  75

GTT GCC TGC TTG GAC ATC TAT TGG ACC GTT CAC CGT GCC CGC AGC CTT        445
Val Ala Cys Leu Asp Ile Tyr Trp Thr Val His Arg Ala Arg Ser Leu
 80                  85                  90                  95

GGT AAC TAT GAG CTG GAT GTC TCC CCC TAT GAA GAC ACA GTG ACC AGC        493
Gly Asn Tyr Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser
                100                 105                 110

AAA CCC TGG AAA ATG AAT CTC AGC AAA CTG AAC ATG CTC AAA CCA GAC        541
Lys Pro Trp Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp
            115                 120                 125

TCA GAC CTC TGC CTC AAG TTT GCC ATG CTG TGT ACT CTC AAT GAC AAG        589
Ser Asp Leu Cys Leu Lys Phe Ala Met Leu Cys Thr Leu Asn Asp Lys
        130                 135                 140

TGT GAC CGG CTG CGC AAG GCC TAC GGG GAG GCG TGC TCC GGG CCC CAC        637
Cys Asp Arg Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Pro His
    145                 150                 155

TGC CAG CGC CAC GTC TGC CTC AGG CAG CTG CTC ACT TTC TTC GAG AAG        685
Cys Gln Arg His Val Cys Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys
160                 165                 170                 175

GCC GCC GAG CCC CAC GCG CAG GGC CTG CTA CTG TGC CCA TGT GCC CCC        733
Ala Ala Glu Pro His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro
                180                 185                 190

AAC GAC CGG GGC TGC GGG GAG CGC CGG CGC AAC ACC ATC GCC CCC AAC        781
Asn Asp Arg Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn
            195                 200                 205

TGC GCG CTG CCG CCT GTG GCC CCC AAC TGC CTG GAG CTG CGG CGC CTC        829
Cys Ala Leu Pro Pro Val Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu
        210                 215                 220

TGC TTC TCC GAC CCG CTT TGC AGA TCA CGC CTG GTG GAT TTC CAG ACC        877
Cys Phe Ser Asp Pro Leu Cys Arg Ser Arg Leu Val Asp Phe Gln Thr
    225                 230                 235

CAC TGC CAT CCC ATG GAC ATC CTA GGA ACT TGT GCA ACA GAG CAG TCC        925
His Cys His Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser
240                 245                 250                 255

AGA TGT CTA CGA GCA TAC CTG GGG CTG ATT GGG ACT GCC ATG ACC CCC        973
Arg Cys Leu Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro
                260                 265                 270

AAC TTT GTC AGC AAT GTC AAC ACC AGT GTT GCC TTA AGC TGC ACC TGC       1021
Asn Phe Val Ser Asn Val Asn Thr Ser Val Ala Leu Ser Cys Thr Cys
            275                 280                 285

CGA GGC AGT GGC AAC CTG CAG GAG GAG TGT GAA ATG CTG GAA GGG TTC       1069
Arg Gly Ser Gly Asn Leu Gln Glu Glu Cys Glu Met Leu Glu Gly Phe
```

290 295 300

TTC TCC CAC AAC CCC TGC CTC ACG GAG GCC ATT GCA GCT AAG ATG CGT 1117
Phe Ser His Asn Pro Cys Leu Thr Glu Ala Ile Ala Ala Lys Met Arg
305 310 315

TTT CAC AGC CAA CTC TTC TCC CAG GAC TGG CCA CAC CCT ACC TTT GCT 1165
Phe His Ser Gln Leu Phe Ser Gln Asp Trp Pro His Pro Thr Phe Ala
320 325 330 335

GTG ATG GCA CAC CAG AAT GAA AAC CCT GCT GTG AGG CCA CAG CCC TGG 1213
Val Met Ala His Gln Asn Glu Asn Pro Ala Val Arg Pro Gln Pro Trp
340 345 350

GTG CCC TCT CTT TTC TCC TGC ACG CTT CCC TTG ATT CTG CTC CTG AGC 1261
Val Pro Ser Leu Phe Ser Cys Thr Leu Pro Leu Ile Leu Leu Leu Ser
355 360 365

CTA TGG TAGCTGGACT TCCCCAGGGC CCTCTTCCCC TCCACCACAC CCAGGTGGAC 1317
Leu Trp

TTGCAGCCCA CAAGGGGTGA GGAAAGGACA GCAGCAGGAA GGAGGTGCAG TGCGCAGATG 1377

AGGGCACAGG AGAAGCTAAG GGTTATGACC TCCAGATCCT TACTGGTCCA GTCCTCATTC 1437

CCTCCACCCC ATCTCCACTT CTGATTCATG CTGCCCCTCC TTGGTGGCCA CAATTTAGCC 1497

ATGTCATCTG GTGGTGACCA GCTCCACCAA GCCCCTTTCT GAGCCCTTCC TCTTGACTAC 1557

CAGGATCACC AGAATCTAAT AAGTTAGCCA TTCTCTATTG CATTCCAGAT TAGGGTTAGG 1617

GTAGGGAGGA CTGGGTGTTC TGAGGCAGCC TAGAAAGTCA TTCTCCTTTG TGAAGAAGGC 1677

TCCTGCCCCC TCGTCTCCTC CTCTGAGTGG AGGATGGAAA ACTACTGCCT GCACTGCCCT 1737

GTCCCCGGAT CCTGCCGAAC ATCTGGGCAT CAGGAGCTGG AGCCTGTGGG CCTTGCTTTA 1797

TTCCTATTAT TGTCCTAAAG TCTCTCTGGG CTCTTGGATC ATGATTAAAC CTTTGACTTA 1857

AAAAAAAAAA AAAAAAAAAA A 1878

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Val Arg Pro Leu Asn Pro Arg Pro Leu Pro Pro Val Val Leu Met
-31 -30 -25 -20

Leu Leu Leu Leu Leu Pro Pro Ser Pro Leu Pro Leu Ala Ala Gly Asp
-15 -10 -5 1

Pro Leu Pro Thr Glu Ser Arg Leu Met Asn Ser Cys Leu Gln Ala Arg
5 10 15

```
Arg Lys Cys Gln Ala Asp Pro Thr Cys Ser Ala Ala Tyr His His Leu
        20                  25                  30

Asp Ser Cys Thr Ser Ser Ile Ser Thr Pro Leu Pro Ser Glu Glu Pro
    35                  40                  45

Ser Val Pro Ala Asp Cys Leu Glu Ala Ala Gln Gln Leu Arg Asn Ser
 50                 55                  60                      65

Ser Leu Ile Gly Cys Met Cys His Arg Arg Met Lys Asn Gln Val Ala
                70                  75                  80

Cys Leu Asp Ile Tyr Trp Thr Val His Arg Ala Arg Ser Leu Gly Asn
            85                  90                  95

Tyr Glu Leu Asp Val Ser Pro Tyr Glu Asp Thr Val Thr Ser Lys Pro
        100                 105                 110

Trp Lys Met Asn Leu Ser Lys Leu Asn Met Leu Lys Pro Asp Ser Asp
    115                 120                 125

Leu Cys Leu Lys Phe Ala Met Leu Cys Thr Leu Asn Asp Lys Cys Asp
130                 135                 140                     145

Arg Leu Arg Lys Ala Tyr Gly Glu Ala Cys Ser Gly Pro His Cys Gln
                150                 155                 160

Arg His Val Cys Leu Arg Gln Leu Leu Thr Phe Phe Glu Lys Ala Ala
            165                 170                 175

Glu Pro His Ala Gln Gly Leu Leu Leu Cys Pro Cys Ala Pro Asn Asp
        180                 185                 190

Arg Gly Cys Gly Glu Arg Arg Arg Asn Thr Ile Ala Pro Asn Cys Ala
    195                 200                 205

Leu Pro Pro Val Ala Pro Asn Cys Leu Glu Leu Arg Arg Leu Cys Phe
210                 215                 220                     225

Ser Asp Pro Leu Cys Arg Ser Arg Leu Val Asp Phe Gln Thr His Cys
                230                 235                 240

His Pro Met Asp Ile Leu Gly Thr Cys Ala Thr Glu Gln Ser Arg Cys
            245                 250                 255

Leu Arg Ala Tyr Leu Gly Leu Ile Gly Thr Ala Met Thr Pro Asn Phe
        260                 265                 270

Val Ser Asn Val Asn Thr Ser Val Ala Leu Ser Cys Thr Cys Arg Gly
    275                 280                 285

Ser Gly Asn Leu Gln Glu Glu Cys Glu Met Leu Glu Gly Phe Phe Ser
290                 295                 300                     305

His Asn Pro Cys Leu Thr Glu Ala Ile Ala Ala Lys Met Arg Phe His
                310                 315                 320

Ser Gln Leu Phe Ser Gln Asp Trp Pro His Pro Thr Phe Ala Val Met
            325                 330                 335

Ala His Gln Asn Glu Asn Pro Ala Val Arg Pro Gln Pro Trp Val Pro
        340                 345                 350
```

```
Ser Leu Phe Ser Cys Thr Leu Pro Leu Ile Leu Leu Leu Ser Leu Trp
    355                 360                 365
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
CTTCTGCCTC TTCTTCTTTC TAGACGAGAC CCTCCGCTCT TTGGCCAGCC CTTCCTCCCT      60

GCAGGGCCCC GAGCTCCACG GCTGGCGCCC CCCAGTGGAC TGTGTCGGGC CAATNAGCTG     120

TGTGCCGCCG AATCCAACTG CAGCTCTCGC TACCGNACTC TGCGGCAGTG CCTGGCAGGN     180

CGCGACCGAA ACACCATGCT GNCAACAAGG AGTTCCAGGG GGCTT                     225
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 364 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GGCANAGNAA TCAGACCTGT GTCCTCACTG TGNGTTGTAA CAGGGTCAGG GGCAGACCCG      60

GTGGTCAGCG CCAAGAGCAA CCATTGCCTG GATGCTGCCA AGGCCTGCAA CCTGAATGAC     120

AACTGCAGAA GCTGCGCTCC TCCTACATCT CCATCTGCAA CCGCGAGATC TCGCCCACCG     180

AGCGCTGCAA CCGCCGCAAG TGCCACAAGG CCCTGCGCCA TTACTTCGAC CGGGTGTCCA     240

GCGNAGTACA CCTTACCGNA TGGTTCTTCT GNTTCCTGCC AAGANCCAGG TGTGCGNTTG     300

AGCCCGCGGG GCAAAACCAT CCTGTNCCAG TTGGTTCCTN TTGAGGACAA GGAGAGGCCC     360

AATT                                                                  364
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAGCCCCACT GGCATCGTGG TGTCCCCCTG GTGCAGCTGT CGTGGNAGCG GGAACATGGA 60

GGAGGAGTGT NAGAAGTTCC TCAGGGACTT CACCGAGAAC CCATGCCTCC GGAACGCCAT 120

CCAGGCCTTT GGCAACGGCA CGGACGTNAA CGTNTCCCCA AAAGGCCCCT CGTTCCAGGC 180

CACCCAGGCC CCTCGGNTGG AGAAGACGCC TTCTTTNCCA GATGACCTCA GTNACAGTAC 240

CAGCTTGGGG ACCAGTGTCA TCACCACCTG CACGTTTNTC CAGGAGCAGG GGCTGAAGGC 300

AACAACTCCA AAGAGTTAAG CATGTNCTTN ACAGAGCTCA CGGCAAATAT 350

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 229 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCAAGAGCAA CCATTGCCTG GATGCTGCCA AGGCCTGCAA CCTGAATGAC AACTGCAAGA 60

AGCTGCGCTC CTCCTACATC TCCATCTGCA ACCGCGAGAT CTCGCCCACC GAGCGCTGCA 120

ACCGCCGCAA GTGCCACAAG GCCCTGCGCC AGTTCTTCGA CCGGGTGCCC AGCGAGTACA 180

CCTACCGCAT GCTCTTCTGC TCCTGCCAAG ATCAGGCGTG CGCTGAGNC 229

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 521 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAACCATTGC CTGGGATGCT GCCAAGGCCT GCAACCTGAA TGACAACTGC AAGAAGCTGC 60

GCTCCTCCTA CATCTCCATC TGCAACCGCG AGATCTCGCC CACCGAGCGC TGCAACCGCC 120

GCAAGTGCCA CAAGGCCCTG CGCCAGTTCT TCGACCGGGT GCCCAGCGAG TACACCTACC 180

GCATGCTCTT CTGCTCCTGC CAAGACCAGG CGTGCGCTGA GCCGCGGNCA AAACCATCCT 240

GCCCAGCTGC TCCTATGAGG ACAAGGAGAA GCCCAACTGC CTGGGACCTG CGTGGCGTGT 300

GCCGGGACTG ACCACCTGTG TCGGTCCCGG CTNGGCCGAC TTTCCATGGC CAATTTGTTG 360

GAGCCTTCCT ACCAGACGGG TCANCAGGTT GCCTTGCGGA CAATTTACCA GGGGTNTTTT 420

GGGGTTTTTA TTGTTGGGCA TGGATTGGGG TTTTGAAATT GANAATTAAT TTTGTTGGGA 480

TTTNCAGGCC CCATTGGGCN TTGTNCCTGN TTCCCCTGGG G 521

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 478 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGACACCTAA CTATGTGGAC TCCAGCCCCA CTGGCATCGT GGTGTCCCCC TGGTGCAGCT 60

GTCGTGGCAG CGGGAACATG GAGGAGGAGT GTGAGAAGTT CCTCAGGGAC TTCACCGAGA 120

ACCCATGCCT CCGGAACGCC ATCCAGGCCT TTGNAACGGC ACGGACGTGA ACGTGTCCCC 180

AAAAGGCCCC TCGTTCCAGG CCACCCAGGC CCTCGGGTGG AGAAGACGCC TTCTTTGCCA 240

GATGACCTCA GTGACAGTAC CAGCTTGGGG ACCAGTGTCA TCACCACCTG CACGTCTGTC 300

CAGGAGCAGG GGCTGAAGGC CAACAACTCC AAAGAGTTAA GCATGTGCTT CACAGAGCTC 360

ACCGACAAAT ATCATCCCAG GGAGTAACAA GGTGATTCAA ACCTAACTCA GGCCCCAGCA 420

GAGCAAGACC GTCGGCTTGC CTTTGACCGT GCTGTCTGTC CTGATGCTGA ACAGGCTT 478

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 433 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAAGGTGTG TGTGTGTCTG TGTGTGTTTC CATTTCGTCA GGCGGCTGTT CTTGTCTGCG 60

TACTTTCAAA AATCTTCTGA CTCGGTTCCC ACAGCCTACA AGGCCTGTTT CAGCATCAGG 120

ACAGACAGCA CGGTCAAGGC AGCCGACGGT CTGGCTCTGC TGGGGCCTGA GTTAGGTTTG 180

ATCACCTTGT TACTCCCTGG GATGATATTT GTCGTGAGCT CTGTGAAGCA CATGCTTAAC 240

TCTTTGGAGT TGTTGGCCTT CAGCCCCTGC TCCTGGACAG ACGTGCAGGT GGTGATGACA 300

CTGGGTCCCC AAGCTGGTAC TGTCACTGAG GTCATCTGGC AAAGAAGGCG TCTTCTCCAC 360

CCGAGGGGCC TGGGGTGGCT GGGAACGAGG GGGCCTTTTT GGGGGACACG TTCACGTTCC 420

GTTGCCGTTG CCA 433

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 418 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTTTTTTTTT TGGGAAAAAC AATTTTTTTT TTGCAAGGTG TGTGTGTGTC TGTGTGTGTT 60

TCCATTTCGT CAGGCGGCTG TTCTTGTCTG CGTANTTTTC AAAAATCTTC TGACTCGGTT 120

CCCACAGCCT ACAAGGCCTG TTTCAGCATC AGGACAGACA GCACGGTCAA GGCAGCCGAC 180

GGTCTGGCTC TGCTGGGGCC TGAGTTAGGT TTGATCACCT TGTTACTCCC TGGGATGATA 240

TTTNTCGTGA GCTCTGTGAA GCACATGCTT AACTCTTTGG AGTTNTTGGC CTTCAGCCCC 300

TGCTCCTGGG ACAGAACGTG CAGGNTGGGT GATGACACTG GGNCCCCAAG GCTGGGTACT 360

GTCACTGAGG GTCATCTGGN CAAAGNAAGG NCGTTTTTCT CCACCCGAGG GGCCGGGG 418

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 364 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGTGTGTGTC TGTGTGTGTT TCCATTTCGT CAGGCGGCTG TTCTTGTCTG CGTAGTTTCA 60

AAAATCTTCT GACTCGGTTC CCACAGCCTA CAAGGNCTGT TTCAGCATCA GGACAGACAG 120

CACGGTCAAG GCAGCCGACG GTCTGGCTCT GCTGGGGCCT GAGTTAGGTT TGATCACCTT 180

GTTACTCCCT GGGATGATAT TTGTCGTGAG CTCTGTGAAG CACATGCTTA ACTCTTTGGA 240

GTTGTTGGCC TTCAGCCCCT GCTCCTGGAC AGACGTGCAG GTGGTNATGA CACTGGTCCC 300

CAAGCTGGTA CTNTCACTGA GGTCATCTGG CAAAGAAGGC GTCTTCTCCA CCCNAGGGGC 360

CTGG 364

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 319 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGAAAAACA ATTTTATTTT TGCAAGGTGT GTGTGTGTCT GTGTGTGTTT CCATTTCGTC 60

AGGCGGCTGT CCTTGTCTGC GTAGTTTCAA AAATCTTCTG ACTCGGTTCC CACAGCCTAC 120

AAGGCCTGTA TAAGCATCAG GACAGACAGC ACGGTCAAGG CAGCCGACGG TCTGGCTCTG 180

CTGGGGCCTG AGTAAGGTTT GNCCACCTTG TAACTCCCTG GGATGATATT TGTCGTGAGC 240

NCTGTNANGC ACATGNTTAA CTCTTTGGAG TTNTTGGCCT TCAGCCCCTG CCCCTGGNCA 300

GACGTGCAGG TGGTGATGA 319

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 309 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCTGAAACTG GCCTTGTAGG CTGTGGGAAC CGAGTCAGAA TATTTTTGAA AGCTACGCAG 60

ACAAGAACNG CGGCCTGACG AAATGGAAAC ACACACAGAC ACACACACNC CTTGCATAAA 120

AAAAATTGTT TTTCCCACCT TGTCGCTGAA CCTGTCTCCT CCCAGGTTTC TTCTCTGGAG 180

AAGTTTTTGT AAACCAAACA GACAAGCAGG CAGGCAGCCT GAGAGCTGGC CCAGGGGTCC 240

CCTGGTCAGG GGAAACTCTG GTGCCGGGGA GGGCACGTGG CTCTAGAAAT GCCCTTCACT 300

TTCTCCTGG 309

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATTCGGCAC AGCCCCTTCC CACAGAAAGC CGACTCATGA ACAGCTGTCT CCAGGCCAGG 60

AGGAAGTGCC AGGCTGATCC CACCTGCAGT GCTGCCTACC ACCACCTGGG ATTTCTGGCA 120

CCTNTAGCAT AAGCACCCCA CTGNCCCTCA GAGGAGNGCT TCGGTCCCTG CTGNACTGCC 180

TGGGGGCACT ACAGNAACTT CAGGAACAGC TTCTNTGATN AGGNTGGCAT GTGCCACCGG 240

GGCATNNTAG TAACCAGGTT TGCCTGCTTG GGACATCTAT TGNGACCGTT NCACCGTTGT 300

CCGGNAGCNT TGGTGAACTN TGTAGCTGGA TGTCTTNCCC CTATGTAAGG TCACAGTGGA 360

CCAGGNAAAA NCCTNGGNAA AAGGAATTTT TCAGCANATT NGNAACCATG ATTCAANANC 420

AGGNNTTNGA NCNCGTTGGG CTTCAAGTTT TTTNCATGGT GGTGTNACTG TGAANNGNNA 480

AGTGTNGGAN C 491

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 368 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCACGAGGT CCAGATGTCT ACGAGCATAC CTGGGGCTGA TTGGGACTGC CATGACCCCC 60

AACTTTNTCA GCAATGTCAA CACCAGTNTT GCCTTAAGCT GCACCTGCCG AGGCATGGCA 120

ACCTGCAGGT AGGAGTGTGA AATGCTGGNA AGGGTTCTTC TCCCACAACC CNTGCCTCAC 180

GGTGGCCATT GCAGCTTAAG AATGCGTTTT CACAGCCAAC TCTTCTGCCC AGGGACTGGC 240

CACACCCTTA CCTTTGGCTT GTGAATGGGC ACACCAGNAA TGGGAAAACC CCTGGCTGTG 300

AAGGGCCANA GNCCTTNGGT GNCCCTGTGT TTTGTTCCTG GCAAGGTTTC CCTTAGGATT 360

TCTGGGTT 368

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 450 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAAGTCAAAG GTTTAATCAT GATCCAAGAG CCCAGAGAGA CTTTAGGACA ATAATAGGAA 60

TAAAGCAAGG CCCACAGGCT CCAGCTCCTG ATGCCCAGAT GTTCGGCAGG ATCCGGGGAC 120

AGGGCAGTGC AGGCAGTTGT TTTCCATCCT CCACTCAGAG GAGGAGACGA GGGGGCAGGA 180

GCTTCTTCAC AAAGGAGAAT GACTTTCTAG GCTGCCTCAG AACACCCAGT CCTCCCTACC 240

CTAACCCTAA TCTGGAATGC AATAGAGAAA GGCTAACTTA TTAGATTCTG GTGATCCTGG 300

TAGTCAAGAG GAAGGGCTCA GAAAGGGGCT TGGTGGAGCT GGTCACCACC AGATGGACAT 360

GGCTAAATTG TGGCCACCAA GGAGGGGCAG CATNAATCAG AAGTGGAGAT GGGGTGGAGG 420

GAATGANGAC TGGACCAGTA AGGATCTGGN 450

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGTGTGCCAT CACAGCAAAG GTAGGGTGTG GCCAGTCCTG GGAGAAGCTA AGGGTTATGA 60

CCTCCAGATC CTTACTGGTC CAGTCCTCAT TCCCTCCACC CCATGTCCAC TTCTGATTCA 120

TGCTGCCCCT CCTTGGTGGC CACAATTTAG CCATGTCATC TGGTGGTGAC CAGCTCCACC 180

AAGCCCCTTT CTGAGCCCTT CCTCTTGACT ACCAGGATCA CCAGAATCTA ATAAGTTAGC 240

CTTTCTCTAT TGCATTCCAG ATTAGGGTTA GGGTAGGGAG GACTGGGTGT TCTGAGGCAG 300

CCTAGAAAGT CATTCTCCTT TGTGAAGAAG GCTCCTGCCC CCTCCGTCTC CTCCTCTTNA 360

GTGGGAGGAT GGAAAACAAC TGCCTGCACT GCCCTTGTCC CCGGATCCTG CCGAACATCT 420

GGGGCATCAG GAAGCT 436

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCATGGCCAG CCCTTCCTCC CTG 23

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGCAAGCTTT TACGGTCTGG CTCTGCTG 28

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGCGGATCCG CCATCATGAT CTTGGCAAAC GTC 33

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGCGGTACCT TACGGTCTGG CTCTGCTGG 29

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGCGGTACCG CAAGGTGTGT GTGTGTC 27

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGCGGATCCG CCATCATGAT CTTGGCAAAC GTC 33

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGCTCTAGAT CAAGCGTAGT CTGGGACGTC GTATGGGTAC GGTCTGGCTC TGCTGG 56

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCAGCAGCCA TGGACCCCCT TCCCACAGAA AGCCGACTCA TGAAC 45

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCAGCAAGAT CTCCATAGGC TCAGGAGCAG AATCAAGGGA AG 42

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GCAGCAAGAT CTCCAGGGCT GTGGCCTCAC AGCAGGGTTT TC 42

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GCACGCGGAT CCGCCATCAT GGTGCGCCCC CTGAACCCGC GACCGCTGCC GCCCGTAGTC 60

CTGATGTTGC TGCTGCTGCT GCCGCCGTCG CCGCTGCCTC TCGCAGCCGG AGACCCCCTT 120

CCCACAGAAA GCCGACTCAT G 141

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCAGCAGGTA CCCTACCATA GGCTCAGGAG CAGAATCAAG GGAAG 45

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCAGCAGGTA CCCTACCAGG GCTGTGGCCT CACAGCAGGG TTTTC 45

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCAGCATCTA GATCAAGCGT AGTCTGGGAC GTCGTATGGG TACCAGGGCT GTGGCCTCAC 60

AGCAGGGTTT TC 72

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCAGCAAGAT CTCTACCATA GGCTCAGGAG CAGAATCAAG GGAAG 45

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCAGCAAGAT CTCTACCAGG GCTGTGGCCT CACAGCAGGG TTTTC 45

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GCAGCAGGAT CCGCCATCAT GGTGCGCCCC CTGAACCCGC GACCGCTG 48

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GCACGCGGAT CCGCCACCAT GGTGCGCCCC CTGAACCCGC GACCGCTG 48

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 7, line 14.	
B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet ☐
Name of depositary institution American Type Culture Collection	
Address of depositary institution *(including postal code and country)* 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit February 14, 1997	Accession Number ATCC 97883
C. ADDITIONAL INDICATIONS *(leave blank if not applicable)*	This information is continued on an additional sheet ☐
DNA Plasmid HSSAE30	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*	
E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*	
The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications, e.g., "Accession Number of Deposit")*	

For receiving Office use only	For International Bureau use only
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Authorized officer Lydell Meadows Paralegal Specialist IAPD-PCT Operations (703) 305-3745	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 7, line 25.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
May 16, 1997	ATCC 209051

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

DNA Plasmid HETDK21X

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications, e.g., "Accession Number of Deposit")*

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Authorized officer ~~Lydell Meadows~~ . aralegal Specialist IAPD-PCT Operations (703) 305-3745	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 8, line 9.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution *(including postal code and country)*

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
May 16, 1997	ATCC 209052

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

DNA Plasmid HETDK21501XX

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications, e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
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Authorized officer Lydell Meadows Paralegal Specialist IAPD-PCT Operations (703) 305-3745	Authorized officer

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the GDNFR-β receptor having the complete amino acid sequence at positions from about -21 to about 443 in SEQ ID NO:2;

(b) a nucleotide sequence encoding the GDNFR-β receptor having the amino acid sequence at positions from about -20 to about 443 in SEQ ID NO:2;

(c) a nucleotide sequence encoding the mature GDNFR-β receptor having the amino acid sequence at positions from about 1 to about 443 in SEQ ID NO:2;

(d) a nucleotide sequence encoding the GDNFR-β receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883;

(e) a nucleotide sequence encoding the mature GDNFR-β receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883;

(f) a nucleotide sequence encoding the GDNFR-β extracellular domain;

(g) a nucleotide sequence encoding the GDNFR-β transmembrane domain;

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 1 (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the GDNFR-β receptor having the complete amino acid sequence in Figure 1 (SEQ ID NO:2).

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the mature GDNFR-β receptor having the amino acid sequence in Figure 1 (SEQ ID NO:2).

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 97883.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the GDNFR-β receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature GDNFR-β receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883.

8. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

9. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a GDNFR-β receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1.

10. The isolated nucleic acid molecule of claim 9, which encodes an epitope-bearing portion of a GDNFR-β receptor selected from the group consisting of:a polypeptide comprising amino acid residues from about 2 to about 59 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 68 to about 85 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 98 to about 116 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 128 to about 200 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 205 to about 270 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 289 to about 374 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 411 to about 428 in SEQ ID NO:2.

11. The isolated nucleic acid molecule of claim 1, which encodes the GDNFR-β receptor extracellular domain.

12. The isolated nucleic acid molecule of claim 1, which encodes the GDNFR-β receptor transmembrane domain.

13. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

14. A recombinant vector produced by the method of claim 13.

15. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 14 into a host cell.

16. A recombinant host cell produced by the method of claim 15.

17. A recombinant method for producing a GDNFR-β polypeptide, comprising culturing the recombinant host cell of claim 16 under conditions such that said polypeptide is expressed and recovering said polypeptide.

18. An isolated GDNFR-β polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the GDNFR-β polypeptide having the complete amino acid sequence at positions from about -21 to about 443 in SEQ ID NO:2;

(b) the amino acid sequence of the GDNFR-β polypeptide having the amino acid sequence at positions from about -20 to about 443 in SEQ ID NO:2;

(c) the amino acid sequence of the mature GDNFR-β polypeptide having the amino acid sequence at positions from about 1 to about 443 in SEQ ID NO:2;

(d) the amino acid sequence of the GDNFR-β polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883;

(e) the amino acid sequence of the mature GDNFR-β polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883;

(f) the amino acid sequence of the GDNFR-β receptor extracellular domain;

(g) the amino acid sequence of the GDNFR-β receptor transmembrane domain; and

(h) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), or (g).

19. An isolated polypeptide comprising an epitope-bearing portion of the GDNFR-β receptor protein, wherein said portion is selected from the group consisting of:a polypeptide comprising amino acid residues from about 2 to about 59 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 68 to about 85 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 98 to about 116 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 128 to about 200 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 205 to about 270 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 289 to about 374 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 411 to about 428 in SEQ ID NO:2.

20. An isolated antibody that binds specifically to a GDNFR-β receptor polypeptide of claim 18.

21. A method of treating diseases and disorders associated with the decreased GDNFR-β activity comprising administering an effective amount of the polypeptide as claimed in claim 18, or an agonist thereof to a patient in need thereof.

22. A method of treating diseases and disorders associated with increased GDNFR-β activity comprising administering an effective amount an antagonist of the polypeptide as claimed in claim 18 to a patient in need thereof.

23. An isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the nucleotide sequence of clone HSSAE30R (SEQ ID NO: 8);

(b) the nucleotide sequence of clone HTLBC22R (SEQ ID NO: 9);

(c) the nucleotide sequence of clone HIBCK30R (SEQ ID NO: 10);

(d) the nucleotide sequence of a portion of the sequence shown in Figure 1 (SEQ ID NO:1) wherein said portion comprises at least 50 contiguous nucleotides from nucleotide 1 to nucleotide 460 or from nucleotide 840 to nucleotide 940; and

(e) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), or (d) above.

24. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the GDNFR-γ1 receptor having the complete amino acid sequence at positions from about -31 to about 347 in (SEQ ID NO:5);

(b) a nucleotide sequence encoding the GDNFR-γ1 receptor having the amino acid sequence at positions from about -30 to about 347 in (SEQ ID NO:5);

(c) a nucleotide sequence encoding the mature GDNFR-γ1 receptor having the amino acid sequence at positions from about 1 to about 347 in (SEQ ID NO:5);

(d) a nucleotide sequence encoding the GDNFR-γ1 receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051;

(e) a nucleotide sequence encoding the mature GDNFR-γ1 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051;

(f) a nucleotide sequence encoding the GDNFR-γ1 extracellular domain;

(g) a nucleotide sequence encoding the GDNFR-γ1 transmembrane domain;

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

25. The nucleic acid molecule of claim 24 wherein said polynucleotide has the complete nucleotide sequence in Figure 4 (SEQ ID NO:4).

26. The nucleic acid molecule of claim 24 wherein said polynucleotide has the nucleotide sequence in Figure 4 (SEQ ID NO:4) encoding the GDNFR-γ1 receptor having the complete amino acid sequence in Figure 4 (SEQ ID NO:5).

27. The nucleic acid molecule of claim 24 wherein said polynucleotide has the nucleotide sequence in Figure 4 (SEQ ID NO:4) encoding the mature GDNFR-γ1 receptor having the amino acid sequence in Figure 4 (SEQ ID NO:5).

28. The nucleic acid molecule of claim 24 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 209051.

29. The nucleic acid molecule of claim 24 wherein said polynucleotide has the nucleotide sequence encoding the GDNFR-γ1 receptor having the complete amino acid sequence encoded by the cDNA clone contained in Deposit No. 209051.

30. The nucleic acid molecule of claim 24 wherein said polynucleotide has the nucleotide sequence encoding the mature GDNFR-γ1 receptor having the

amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051.

31. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), or (h) of claim 24 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

32. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a GDNFR-γ1 receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f), (g), or (h) of claim 24.

33. The isolated nucleic acid molecule of claim 32, which encodes an epitope-bearing portion of a GDNFR-γ1 receptor selected from the group consisting of: a polypeptide comprising amino acid residues from about 2 to about 10 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 13 to about 26 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 33 to about 40 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 42 to about 56 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 59 to about 67 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 71 to about 77 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 90 to about 114 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 122 to about 129 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 139 to about 164 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 174 to about 180 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 187 to about 203 in SEQ ID NO:5; a polypeptide

comprising amino acid residues from about 217 to about 235 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 250 to about 257 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 302 to about 307 in SEQ ID NO:5; and a polypeptide comprising amino acid residues from about 317 to about 325 in SEQ ID NO:5.

34. The isolated nucleic acid molecule of claim 24, which encodes the GDNFR-γ1 receptor extracellular domain.

35. The isolated nucleic acid molecule of claim 24, which encodes the GDNFR-γ1 receptor transmembrane domain.

36. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 24 into a vector.

37. A recombinant vector produced by the method of claim 36.

38. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 37 into a host cell.

39. A recombinant host cell produced by the method of claim 38.

40. A recombinant method for producing a GDNFR-γ1 polypeptide, comprising culturing the recombinant host cell of claim 39 under conditions such that said polypeptide is expressed and recovering said polypeptide.

41. An isolated GDNFR-γ1 polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the GDNFR-γ1 polypeptide having the complete amino acid sequence at positions from about -31 to about 347 in (SEQ ID NO:5);

(b) the amino acid sequence of the GDNFR-γ1 polypeptide having the amino acid sequence at positions from about -30 to about 347 in (SEQ ID NO:5);

(c) the amino acid sequence of the mature GDNFR-γ1 polypeptide having the amino acid sequence at positions from about 1 to about 347 in(SEQ ID NO:5);

(d) the amino acid sequence of the GDNFR-γ1 polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051;

(e) the amino acid sequence of the mature GDNFR-γ1 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209051;

(f) the amino acid sequence of the GDNFR-γ1 receptor extracellular domain;

(g) the amino acid sequence of the GDNFR-γ1 receptor transmembrane domain;

(h) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), or (g).

42. An isolated polypeptide comprising an epitope-bearing portion of the GDNFR-γ1 receptor protein, wherein said portion is selected from the group consisting of:a polypeptide comprising amino acid residues from about 2 to about 10 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 13 to about 26 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 33 to about 40 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 42 to about 56 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 59 to about 67 in SEQ ID NO:5; a polypeptide

comprising amino acid residues from about 71 to about 77 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 90 to about 114 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 122 to about 129 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 139 to about 164 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 174 to about 180 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 187 to about 203 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 217 to about 235 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 250 to about 257 in SEQ ID NO:5; a polypeptide comprising amino acid residues from about 302 to about 307 in SEQ ID NO:5; and a polypeptide comprising amino acid residues from about 317 to about 325 in SEQ ID NO:5.

43. An isolated antibody that binds specifically to a GDNFR-γ1 receptor polypeptide of claim 41.

44. An isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the nucleotide sequence of clone HEKTDK21 (SEQ ID NO: 19);

(b) the nucleotide sequence of a portion of the sequence shown in Figure 4 (SEQ ID NO:4) wherein said portion comprises at least 50 contiguous nucleotides from nucleotide 1 - 1,111, 1-250, 250-500, 500-750, 750-1,111; and

(c) a nucleotide sequence complementary to any of the nucleotide sequences in (a) or (b) above.

45. A method of treating diseases and disorders associated with the decreased GDNFR-γ1 activity comprising administering an effective amount of

the polypeptide as claimed in claim 41, or an agonist thereof to a patient in need thereof.

46. A method of treating diseases and disorders associated with increased GDNFR-γ1 activity comprising administering an effective amount an antagonist of the polypeptide as claimed in claim 41 to a patient in need thereof.

47. An isolated polynucleotide encoding a modified GDNFR-γ1 protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -31 to 347 of SEQ ID NO:5;
(b) amino acids -30 to 347 of SEQ ID NO:5; and
(c) amino acids 1 to 347 of SEQ ID NO:5.

48. A modified GDNFR-γ1 protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -31 to 347 of SEQ ID NO:5;
(b) amino acids -30 to 347 of SEQ ID NO:5; and
(c) amino acids 1 to 347 of SEQ ID NO:5.

49. An isolated polynucleotide encoding a modified GDNFR-β protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -21 to 443 of SEQ ID NO:2;
(b) amino acids -20 to 443 of SEQ ID NO:2; and
(c) amino acids 1 to 443 of SEQ ID NO:2.

50. A modified GDNFR-β protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -21 to 443 of SEQ ID NO:2;

(b) amino acids -20 to 443 of SEQ ID NO:2; and

(c) amino acids 1 to 443 of SEQ ID NO:2.

51. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the GDNFR-γ2 receptor having the complete amino acid sequence at positions from about -31 to about 369 (SEQ ID NO:7);

(b) a nucleotide sequence encoding the GDNFR-γ2 receptor having the amino acid sequence at positions from about -30 to about 369 in (SEQ ID NO:7);

(c) a nucleotide sequence encoding the mature GDNFR-γ2 receptor having the amino acid sequence at positions from about 1 to about 369 in (SEQ ID NO:7);

(d) a nucleotide sequence encoding the GDNFR-γ2 receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052;

(e) a nucleotide sequence encoding the mature GDNFR-γ2 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052;

(f) a nucleotide sequence encoding the GDNFR-γ2 extracellular domain;

(g) a nucleotide sequence encoding the GDNFR-γ2 transmembrane domain;

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

52. The nucleic acid molecule of claim 51 wherein said polynucleotide has the complete nucleotide sequence in Figure 7 (SEQ ID NO:6).

53. The nucleic acid molecule of claim 51 wherein said polynucleotide has the nucleotide sequence in Figure 7 (SEQ ID NO:6) encoding the GDNFR-γ2 receptor having the complete amino acid sequence in Figure 7 (SEQ ID NO:6).

54. The nucleic acid molecule of claim 51 wherein said polynucleotide has the nucleotide sequence in Figure 7 (SEQ ID NO:6) encoding the mature GDNFR-γ2 receptor having the amino acid sequence in Figure 7 (SEQ ID NO:6).

55. The nucleic acid molecule of claim 51 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 209052.

56. The nucleic acid molecule of claim 51 wherein said polynucleotide has the nucleotide sequence encoding the GDNFR-γ2 receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052.

57. The nucleic acid molecule of claim 51 wherein said polynucleotide has the nucleotide sequence encoding the mature GDNFR-γ2 receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052.

58. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide

having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), or (h) of claim 51 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

59. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a GDNFR-γ2 receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f), (g), or (h) of claim 51.

60. The isolated nucleic acid molecule of claim 59, which encodes an epitope-bearing portion of a GDNFR-γ2 receptor selected from the group consisting of:a polypeptide comprising amino acid residues from about 1 to about 9 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 14 to about 27 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 34 to about 41 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 43 to about 57 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 60 to about 68 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 72 to about 78 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 91 to about 115 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 122 to about 130 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 140 to about 165 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 175 to about 181 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 189 to about 204 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 216 to about 222 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 224 to about 236 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 251 to about 259 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 285 to about 299 in SEQ ID NO:7; a polypeptide comprising amino acid

residues from about 314 to about 320 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 325 to about 330 in SEQ ID NO:7; and a polypeptide comprising amino acid residues from about 340 to about 348 in SEQ ID NO:7.

61. The isolated nucleic acid molecule of claim 51, which encodes the GDNFR-γ2 receptor extracellular domain.

62. The isolated nucleic acid molecule of claim 51, which encodes the GDNFR-γ2 receptor transmembrane domain.

63. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 51 into a vector.

64. A recombinant vector produced by the method of claim 63.

65. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 64 into a host cell.

66. A recombinant host cell produced by the method of claim 65.

67. A recombinant method for producing a GDNFR-γ2 polypeptide, comprising culturing the recombinant host cell of claim 66 under conditions such that said polypeptide is expressed and recovering said polypeptide.

68. An isolated GDNFR-γ2 polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the GDNFR-γ2 polypeptide having the complete amino acid sequence at positions from about -31 to about 369 (SEQ ID NO:7);

(b) the amino acid sequence of the GDNFR-γ2 polypeptide having the amino acid sequence at positions from about -30 to about 369 in (SEQ ID NO:7);

(c) the amino acid sequence of the mature GDNFR-γ2 polypeptide having the amino acid sequence at positions from about 1 to about 369 in(SEQ ID NO:7);

(d) the amino acid sequence of the GDNFR-γ2 polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052;

(e) the amino acid sequence of the mature GDNFR-γ2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209052;

(f) the amino acid sequence of the GDNFR-γ2 receptor extracellular domain;

(g) the amino acid sequence of the GDNFR-γ2 receptor transmembrane domain;

(h) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), or (g).

69. An isolated polypeptide comprising an epitope-bearing portion of the GDNFR-γ2 receptor protein, wherein said portion is selected from the group consisting of:a polypeptide comprising amino acid residues from about 1 to about 9 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 14 to about 27 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 34 to about 41 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 43 to about 57 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 60 to about 68 in SEQ ID NO:7; a polypeptide

comprising amino acid residues from about 72 to about 78 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 91 to about 115 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 122 to about 130 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 140 to about 165 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 175 to about 181 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 189 to about 204 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 216 to about 222 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 224 to about 236 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 251 to about 259 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 285 to about 299 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 314 to about 320 in SEQ ID NO:7; a polypeptide comprising amino acid residues from about 325 to about 330 in SEQ ID NO:7; and a polypeptide comprising amino acid residues from about 340 to about 348 in SEQ ID NO:7.

70. An isolated antibody that binds specifically to a GDNFR-γ2 receptor polypeptide of claim 68.

71. An isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the nucleotide sequence of clone HEKTDK21 (SEQ ID NO: 19);

(b) the nucleotide sequence of a portion of the sequence shown in Figure 7 (SEQ ID NO:6) wherein said portion comprises at least 50 contiguous nucleotides from nucleotide 1 - 1,111, 1-250, 250-500, 500-750, 750-1,111; and

(c) a nucleotide sequence complementary to any of the nucleotide sequences in (a) or (b) above.

72. A method of treating diseases and disorders associated with the decreased GDNFR-γ2 activity comprising administering an effective amount of the polypeptide as claimed in claim 68, or an agonist thereof to a patient in need thereof.

73. A method of treating diseases and disorders associated with increased GDNFR-γ2 activity comprising administering an effective amount an antagonist of the polypeptide as claimed in claim 68 to a patient in need thereof.

74. An isolated polynucleotide encoding a modified GDNFR-γ2 protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -31 to 369 of SEQ ID NO:7;
(b) amino acids -30 to 369 of SEQ ID NO:7; and
(c) amino acids 1 to 369 of SEQ ID NO:7.

75. A modified GDNFR-γ2 protein, wherein, except for at least one conservative amino acid substitution, said modified protein has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -31 to 369 of SEQ ID NO:7;
(b) amino acids -30 to 369 of SEQ ID NO:7; and
(c) amino acids 1 to 369 of SEQ ID NO:7.

```
  1 GGGAGAAAGACAAAAAAACGGTGGGATTTATTTAACATGATCTTGGCAAACGTCTTCTGC  60
  1                                     M  I  L  A  N  V  F  C   20

 61 CTCTTCTTCTTTCTAGACGAGACCCTCCGCTCTTTGGCCAGCCCTTCCTCCCTGCAGGGC 120
 21 L  F  F  F  L  D  E  T  L  R  S  L  A  S  P  S  S  L  Q  G   40

121 CCCGAGCTCCACGGCTGGCGCCCCCCAGTGGACTGTGTCCGGGCCAATGAGCTGTGTGCC 180
 41 P  E  L  H  G  W  R  P  P  V  D  C  V  R  A  N  E  L  C  A   60

181 GCCGAATCCAACTGCAGCTCTCGCTACCGCACTCTGCGGCAGTGCCTGGCAGGCCGCGAC 240
 61 A  E  S  N  C  S  S  R  Y  R  T  L  R  Q  C  L  A  G  R  D   80

241 CGCAACACCATGCTGGCCAACAAGGAGTGCCAGGCGGCCTTGGAGGTCTTGCAGGAGAGC 300
 81 R  N  T  M  L  A  N  K  E  C  Q  A  A  L  E  V  L  Q  E  S  100

301 CCGCTGTACGACTGCCGCTGCAAGCGGGGCATGAAGAAGGAGCTGCAGTGTCTGCAGATC 360
101 P  L  Y  D  C  R  C  K  R  G  M  K  K  E  L  Q  C  L  Q  I  120

361 TACTGGAGCATCCACCTGGGGCTGACCGAGGGTGAGGAGTTCTACGAAGCCTCCCCCTAT 420
121 Y  W  S  I  H  L  G  L  T  E  G  E  E  F  Y  E  A  S  P  Y  140

421 GAGCCGGTGACCTCCCGCCTCTCGGACATCTTCAGGCTTGCTTCAATCTTCTCAGGGACA 480
141 E  P  V  T  S  R  L  S  D  I  F  R  L  A  S  I  F  S  G  T  160

481 GGGGCAGACCCGGTGGTCAGCGCCAAGAGCAACCATTGCCTGGATGCTGCCAAGGCCTGC 540
161 G  A  D  P  V  V  S  A  K  S  N  H  C  L  D  A  A  K  A  C  180

541 AACCTGAATGACAACTGCAAGAAGCTGCGCTCCTCCTACATCTCCATCTGCAACCGCGAG 600
181 N  L  N  D  N  C  K  K  L  R  S  S  Y  I  S  I  C  N  R  E  200

601 ATCTCGCCCACCGAGCGCTGCAACCGCCGCAAGTGCCACAAGGCCCTGCGCCAGTTCTTC 660
201 I  S  P  T  E  R  C  N  R  R  K  C  H  K  A  L  R  Q  F  F  220

661 GACCGGGTGCCCAGCGAGTACACCTACCGCATGCTCTTCTGCTCCTGCCAAGACCAGGCG 720
221 D  R  V  P  S  E  Y  T  Y  R  M  L  F  C  S  C  Q  D  Q  A  240

721 TGCGCTGAGCGCCGCCGGCAAACCATCCTGCCCAGCTGCTCCTATGAGGACAAGGAGAAG 780
241 C  A  E  R  R  R  Q  T  I  L  P  S  C  S  Y  E  D  K  E  K  260

781 CCCAACTGCCTGGACCTGCGTGGCGTGTGCCGGACTGACCACCTGTGTCGGTCCCGGCTG 840
261 P  N  C  L  D  L  R  G  V  C  R  T  D  H  L  C  R  S  R  L  280

841 GCCGACTTCCATGCCAATTGTCGAGCCTCCTACCAGACGGTCACCAGCTGCCCTGCGGAC 900
281 A  D  F  H  A  N  C  R  A  S  Y  Q  T  V  T  S  C  P  A  D  300
```

FIG. 1A

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901 AATTACCAGGCGTGTCTGGGCTCTTATGCTGGCATGATTGGGTTTGACATGACACCTAAC 960
301 N Y Q A C L G S Y A G M I G F D M T P N 320

961 TATGTGGACTCCAGCCCCACTGGCATCGTGGTGTCCCCCTGGTGCAGCTGTCGTGGCAGC 1020
321 Y V D S S P T G I V V S P W C S C R G S 340

1021 GGGAACATGGAGGAGGAGTGTGAGAAGTTCCTCAGGGACTTCACCGAGAACCCATGCCTC 1080
341 G N M E E E C E K F L R D F T E N P C L 360

1081 CGGAACGCCATCCAGGCCTTTGGCAACGGCACGGATGTGAACGTGTCCCCAAAAGGCCCC 1140
361 R N A I Q A F G N G T D V N V S P K G P 380

1141 TCGTTCCAGGCCACCCAGGCCCCTCGGGTGGAGAAGACGCCTTCTTTGCCAGATGACCTC 1200
381 S F Q A T Q A P R V E K T P S L P D D L 400

1201 AGTGACAGTACCAGCTTGGGGACCAGTGTCATCACCACCTGCACGTCTGTCCAGGAGCAG 1260
401 S D S T S L G T S V I T T C T S V Q E Q 420

1261 GGGCTGAAGGCCAACAACTCCAAAGAGTTAAGCATGTGCTTCACAGAGCTCACGACAAAT 1320
421 G L K A N N S K E L S M C F T E L T T N 440

1321 ATCATCCCAGGGAGTAACAAGGTGATCAAACCTAACTCAGGCCCCAGCAGAGCCAGACCG 1380
441 I I P G S N K V I K P N S G P S R A R P 460

1381 TCGGCTGCCTTGACCGTGCTGTCTGTCCTGATGCTGAAACTGGCCTTGTAGGCTGTGGGA 1440
461 S A A L T V L S V L M L K L A L * 480

1441 ACCGAGTCAGAAGATTTTTGAAATACGCAGACAAGAACAGCCGCCTGACGAAATGGAAAC 1500

1501 ACACACAGACACACACACACCTTGCAAAAAAAAAAAAAAAAAAAA 1543

FIG. 1B

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```
  1  MILANVFCLFFFLDETLRSLASPSSLQGPELHGWRPPVDCVRANELCAAE   50
     |:||.::  : :||  :.. .|.:.           .:|||:|.: |  |
  1  MFLATLYFALPLLDLLMSAEVSGGD...........RLDCVKASDQCLKE   39

 51  SNCSSRYRTLRQCLAGRDRN.....TMLANKECQAALEVLQESPLYDCRC   95
      .||.:|||||||:||:: |    .: |..||..|:|.|.:...||:|||
 40  QSCSTKYRTLRQCVAGKETNFSLTSGLEAKDECRSAMEALKQKSLYNCRC   89

 96  KRGMKKELQCLQIYWSIHLGLTEGEEFYEASPYEPVTSRLSDIFRLASIF  145
     ||||||| .||.||||:. :| :|:::.|.||||||.||||||||  ..::
 90  KRGMKKEKNCLRIYWSMYQSL.QGNDLLEDSPYEPVNSRLSDIFRAVPFI  138

146  SGTGADPVVSAKSNHCLDAAKACNLNDNCKKLRSSYISICNREISPTERC  195
     |:. .:.    .|:|:||||||||||:|.|||.||.||. |. .:| .| |
139  SDVFQQVEHISKGNNCLDAAKACNLDDTCKKYRSAYITPCTTSMS.NEVC  187

196  NRRKCHKALRQFFDRVPSEYTYRMLFCSCQDQACAERRRQTILPSCSYED  245
     ||||||||||||||:||....| ||||||.| ||.|||||||:| ||||:
188  NRRKCHKALRQFFDKVPAKHSYGMLFCSCRDIACTERRRQTIVPVCSYEE  237

246  KEKPNCLDLRGVCRTDHLCRSRLADFHANCRASYQTVTSCPADNYQACLG  295
     :|:||||.|.: |:|:.:||||||||  .||.:. ..|..|  :||..||
238  RERPNCLSLQDSCKTNYICRSRLADFFTNCQPESRSVSNCLKENYADCLL  287

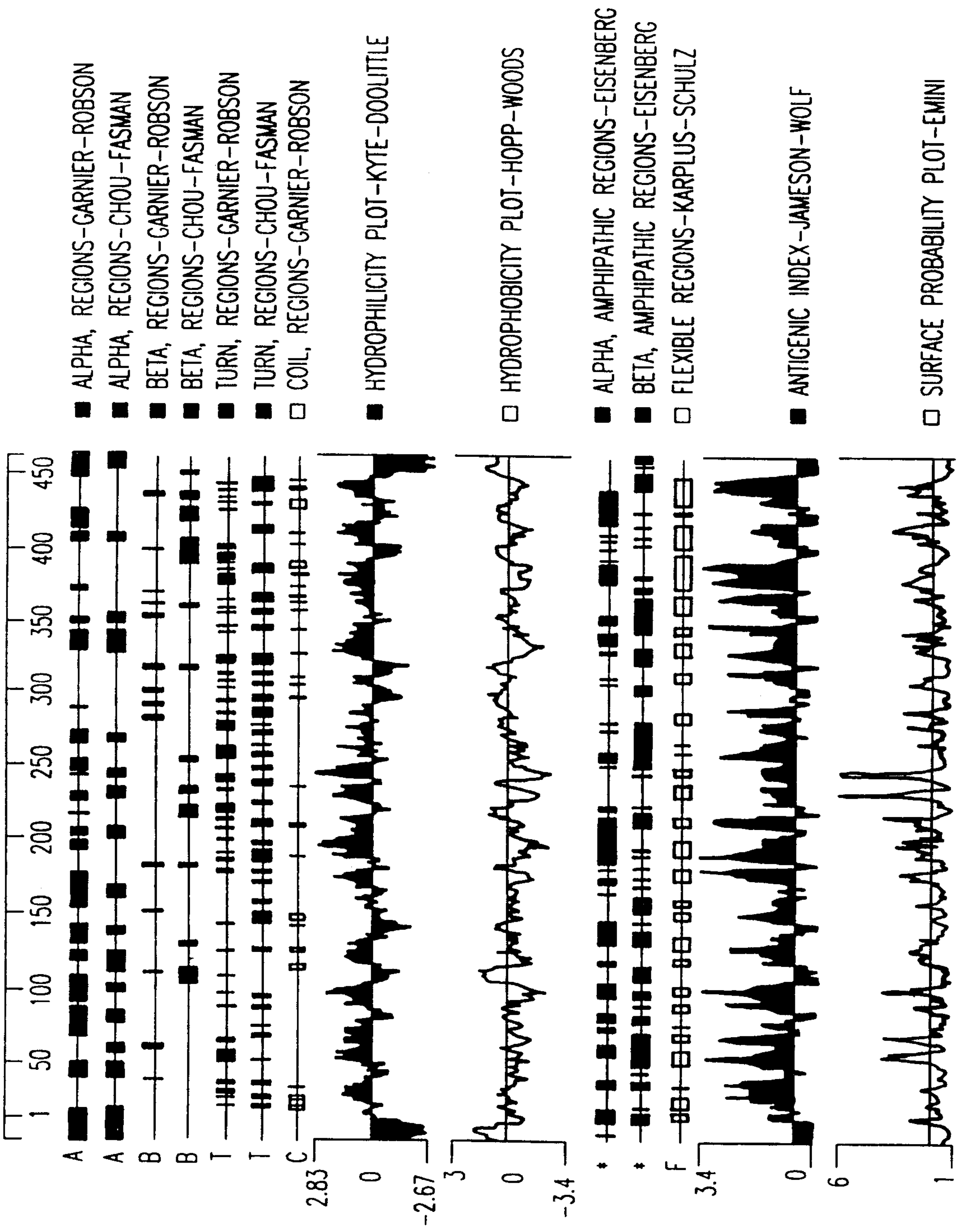
296  SYAGMIGFDMTPNYVDSSPTGIVVSPWCSCRGSGNMEEECEKFLRDFTEN  345
     .|.|:||  |||||||||  :: |.|||.|..|||  |:| |||. |.:|
288  AYSGLIGTVMTPNYVDSS..SLSVAPWCDCSNSGNDLEDCLKFLNFFKDN  335

346  PCLRNAIQAFGNGTDVNVSPKGPSFQATQAPRVEKTPSLPDDLSDSTS..  393
     .||:|||||||||.||.:...:|..|.| |. ..  .   ..|:...|
336  TCLKNAIQAFGNGSDVTMWQPAPPVQTTTATTTTAFRVKNKPLGPAGSEN  385

394  .LGTSVITTCTSVQEQGLKAN..NSKELSMCFTELTTNIIPGSNKVIKPN  440
      :.| |:..|..:|.| ||.|  .|..|:::  .:..: ::|... |...
386  EIPTHVLPPCANLQAQKLKSNVSGSTHLCLSDSDFGKDGLAGASSHITTK  435

441  SGPSRARPSAALTVLSVLMLK  461
     | :  |.||..|. |.||||.
436  SMA..APPSCSLSSLPVLMLT  454
```

FIG.2



ALPHA, REGIONS-GARNIER-ROBSON
ALPHA, REGIONS-CHOU-FASMAN
BETA, REGIONS-GARNIER-ROBSON
BETA, REGIONS-CHOU-FASMAN
TURN, REGIONS-GARNIER-ROBSON
TURN, REGIONS-CHOU-FASMAN
COIL, REGIONS-GARNIER-ROBSON
HYDROPHILICITY PLOT-KYTE-DOOLITTLE
HYDROPHOBICITY PLOT-HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS-EISENBERG
BETA, AMPHIPATHIC REGIONS-EISENBERG
FLEXIBLE REGIONS-KARPLUS-SCHULZ
ANTIGENIC INDEX-JAMESON-WOLF
SURFACE PROBABILITY PLOT-EMINI
1
50
100
150
200
250
300
350
400
450
A
A
B
B
T
T
C
2.83
0
-2.67
3
0
-3.4
*
*
F
3.4
0
6
1

FIG.3

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```
      610                 630                 650
GCGCAAGGCCTACGGGGAGGCGTGCTCCGGGCCCCACTGCCAGCGCCACGTCTGCCTCAG
 R  K  A  Y  G  E  A  C  S  G  P  H  C  Q  R  H  V  C  L  R

      670                 690                 710
GCAGCTGCTCACTTTCTTCGAGAAGGCCGCCGAGCCCCACGCGCAGGGCCTGCTACTGTG
 Q  L  L  T  F  F  E  K  A  A  E  P  H  A  Q  G  L  L  L  C

      730                 750                 770
CCCATGTGCCCCCAACGACCGGGGCTGCGGGGAGCGCCGGCGCAACACCATCGCCCCCAA
 P  C  A  P  N  D  R  G  C  G  E  R  R  R  N  T  I  A  P  N

      790                 810                 830
CTGCGCGCTGCCGCCTGTGGCCCCCAACTGCCTGGAGCTGCGGCGCCTCTGCTTCTCCGA
 C  A  L  P  P  V  A  P  N  C  L  E  L  R  R  L  C  F  S  D

      850                 870                 890
CCCGCTTTGCAGATCACGCCTGGTGGATTTCCAGACCCACTGCCATCCCATGGACATCCT
 P  L  C  R  S  R  L  V  D  F  Q  T  H  C  H  P  M  D  I  L

      910                 930                 950
AGGAACTTGTGCAACAGAGCAGTCCAGATGTCTACGAGCATACCTGGGGCTGATTGGGAC
 G  T  C  A  T  E  Q  S  R  C  L  R  A  Y  L  G  L  I  G  T

      970                 990                1010
TGCCATGACCCCCAACTTTGTCAGCAATGTCAACACCAGTGTTGCCTTAAGCTGCACCTG
 A  M  T  P  N  F  V  S  N  V  N  T  S  V  A  L  S  C  T  C

     1030                1050                1070
CCTCACGGAGGCCATTGCAGCTAAGATGCGTTTTCACAGCCAACTCTTCTCCCAGGACTG
 L  T  E  A  I  A  A  K  M  R  F  H  S  Q  L  F  S  Q  D  W

     1090                1110                1130
GCCACACCCTACCTTTGCTGTGATGGCACACCAGAATGAAAACCCTGCTGTGAGGCCACA
 P  H  P  T  F  A  V  M  A  H  Q  N  E  N  P  A  V  R  P  Q

     1150                1170                1190
GCCCTGGGTGCCCTCTCTTTTCTCCTGCACGCTTCCCTTGATTCTGCTCCTGAGCCTATG
 P  W  V  P  S  L  F  S  C  T  L  P  L  I  L  L  L  S  L  W
```

FIG. 4B

```
        1210                1230                1250
GTAGCTGGACTTCCCCAGGGCCCTCTTCCCCTCCACCACACCCAGGTGGACTTGCAGCCC
*
        1270                1290                1310
ACAAGGGGTGAGGAAAGGACAGCAGCAGGAAGGAGGTGCAGTGCGCAGATGAGGGCACAG

        1330                1350                1370
GAGAAGCTAAGGGTTATGACCTCCAGATCCTTACTGGTCCAGTCCTCATTCCCTCCACCC

        1390                1410                1430
CATCTCCACTTCTGATTCATGCTGCCCCTCCTTGGTGGCCACAATTTAGCCATGTCATCT

        1450                1470                1490
GGTGGTGACCAGCTCCACCAAGCCCCTTTCTGAGCCCTTCCTCTTGACTACCAGGATCAC

        1510                1530                1550
CAGAATCTAATAAGTTAGCCTTTCTCTATTGCATTCCAGATTAGGGTTAGGGTAGGGAGG

        1570                1590                1610
ACTGGGTGTTCTGAGGCAGCCTAGAAAGTCATTCTCCTTTGTGAAGAAGGCTCCTGCCCC

        1630                1650                1670
CTCGTCTCCTCCTCTGAGTGGAGGATGGAAAACTACTGCCTGCACTGCCCTGTCCCCGGA

        1690                1710                1730
TCCTGCCGAACATCTGGGCATCAGGAGCTGGAGCCTGTGGGCCTTGCTTTATTCCTATTA

        1750                1770                1790
TTGTCCTAAAGTCTCTCTGGGCTCTTGGATCATGATTAAACCTTTGACTTAAAAAAAAAA

AAAAAAAAA
```

FIG. 4C

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```
              .         .         .         .         .
 14  VLMLLLLLPPSPLPLAAGDPLPTESRLMNSCLQARRKCQADPTCSAAYHH  63
     :::  |.:: . |.|  :..:..:.||  .|:.|. .|  :..||. |:
  1  MFLATLYFALPLLDLLMSAEVSGGDRL..DCVKASDQCLKEQSCSTKYRT  48

              .         .         .         .         .
 64  LDSCTSSISTPLP.SEEPSVPADCLEAAQQLRNSSLIGCMCHRRMKNQVA  112
     |  |..: .| :. ..: ....:| .| :.|:..||..|.|.| ||.: .
 49  LRQCVAGKETNFSLTSGLEAKDECRSAMEALKQKSLYNCRCKRGMKKEKN  98

              .         .         .         .         .
113  CLDIYWTVHRARSLGNYELDVSPYE.......DTVTSKPWKMNLSKLNML  155
     || |||.:...    ||  |: ||||       |.. . |:  :: .
 99  CLRIYWSMYQS.LQGNDLLEDSPYEPVNSRLSDIFRAVPFISDVFQQVEH  147

              .         .         .         .         .
156  KPDSDLCLKFAMLCTLNDKCDRLRKAYGEAC....SGPHCQRHVCLRQLL  201
      ..:: ||. |. |.|:|.|.:.|.|| .:|    |.. |.|: | :.|
148  ISKGNNCLDAAKACNLDDTCKKYRSAYITPCTTSMSNEVCNRRKCHKALR  197

              .         .         .         .         .
202  TFFEKAAEPHAQGLLLCPCAPNDRGCGERRRNTIAPNCALPPVA.PNCLE  250
      ||:|.:..|. |:|:|.|  .| :|.||||.||.| |.... . ||||.
198  QFFDKVPAKHSYGMLFCSC..RDIACTERRRQTIVPVCSYEERERPNCLS  245

              .         .         .         .         .
251  LRRLCFSDPLCRSRLVDFQTHCHPMD.ILGTCATEQ.SRCLRAYLGLIGT  298
     |.  | .: :|||||.|| |:|:| .  ::.| .|. . || || |||||
246  LQDSCKTNYICRSRLADFFTNCQPESRSVSNCLKENYADCLLAYSGLIGT  295

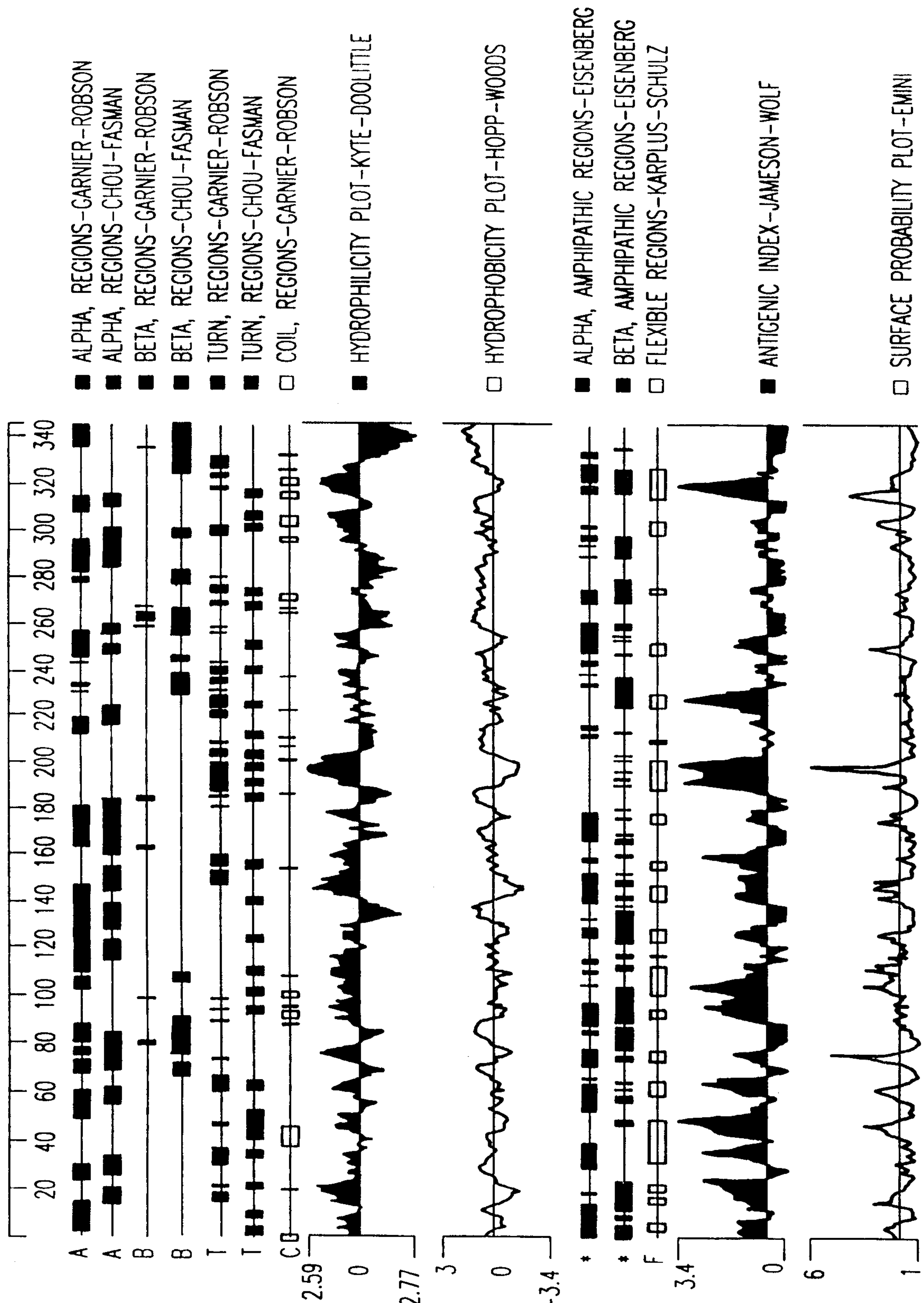
              .         .         .         .         .
299  AMTPNFVSNVNTSVALSC......................TCLTEAI...  323
     .||||:|.. . ||| .|                      |||.:||
296  VMTPNYVDSSSLSVAPWCDCSNSGNDLEDCLKFLNFFKDNTCLKNAIQAF  345

                             .
                             .
                             .

              .         .         .         .         .
324  ....AAKMRFH...........SQLFSQDWPHPTFAVMAHQNENPAVRPQ  358
         |.|:: :           |:: .:::: :.  : ..  ..|:  .
396  ANLQAQKLKSNVSGSTHLCLSDSDFGKDGLAGASSHITTKSMAAPPSCSL  445

359  PWVPSLFSCTLPLILLLSL  377
     ..:| |: ..|: :| :||
446  SSLPVLMLTALAALLSVSL  464
```

FIG.5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TURN, REGIONS-GARNIER-ROBSON
TURN, REGIONS-CHOU-FASMAN
COIL, REGIONS-GARNIER-ROBSON
HYDROPHILICITY PLOT-KYTE-DOOLITTLE
HYDROPHOBICITY PLOT-HOPP-WOODS
ALPHA, AMPHIPATHIC REGIONS-EISENBERG
BETA, AMPHIPATHIC REGIONS-EISENBERG
FLEXIBLE REGIONS-KARPLUS-SCHULZ
ANTIGENIC INDEX-JAMESON-WOLF
SURFACE PROBABILITY PLOT-EMINI
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340
A
A
B
B
T
T
C
2.59
0
-2.77
3
0
-3.4
*
*
F
3.4
0
6
1

FIG.6

```
       10                  30                  50
GAGCCCCGCTCTCAGAGCTCCAGGGGAGGAGCGAGGGGAGCGCGGAGCCCGGCGCCTACA

       70                  90                 110
GCTCGCCATGGTGCGCCCCCTGAACCCGCGACCGCTGCCGCCCGTAGTCCTGATGTTGCT
         M  V  R  P  L  N  P  R  P  L  P  P  V  V  L  M  L  L

      130                 150                 170
GCTGCTGCTGCCGCCGTCGCCGCTGCCTCTCGCAGCCGGAGACCCCCTTCCCACAGAAAG
 L  L  L  P  P  S  P  L  P  L  A  A  G  D  P  L  P  T  E  S

      190                 210                 230
CCGACTCATGAACAGCTGTCTCCAGGCCAGGAGGAAGTGCCAGGCTGATCCCACCTGCAG
 R  L  M  N  S  C  L  Q  A  R  R  K  C  Q  A  D  P  T  C  S

      250                 270                 290
TGCTGCCTACCACCACCTGGATTCCTGCACCTCTAGCATAAGCACCCCACTGCCCTCAGA
 A  A  Y  H  H  L  D  S  C  T  S  S  I  S  T  P  L  P  S  E

      310                 330                 350
GGAGCCTTCGGTCCCTGCTGACTGCCTGGAGGCAGCACAGCAACTCAGGAACAGCTCTCT
 E  P  S  V  P  A  D  C  L  E  A  A  Q  Q  L  R  N  S  S  L

      370                 390                 410
GATAGGCTGCATGTGCCACCGGCGCATGAAGAACCAGGTTGCCTGCTTGGACATCTATTG
 I  G  C  M  C  H  R  R  M  K  N  Q  V  A  C  L  D  I  Y  W

      430                 450                 470
GACCGTTCACCGTGCCCGCAGCCTTGGTAACTATGAGCTGGATGTCTCCCCCTATGAAGA
 T  V  H  R  A  R  S  L  G  N  Y  E  L  D  V  S  P  Y  E  D
```

FIG. 7A

```
       490                 510                 530
CACAGTGACCAGCAAACCCTGGAAAATGAATCTCAGCAAACTGAACATGCTCAAACCAGA
 T  V  T  S  K  P  W  K  M  N  L  S  K  L  N  M  L  K  P  D

       550                 570                 590
CTCAGACCTCTGCCTCAAGTTTGCCATGCTGTGTACTCTCAATGACAAGTGTGACCGGCT
 S  D  L  C  L  K  F  A  M  L  C  T  L  N  D  K  C  D  R  L

       610                 630                 650
GCGCAAGGCCTACGGGGAGGCGTGCTCCGGGCCCCACTGCCAGCGCCACGTCTGCCTCAG
 R  K  A  Y  G  E  A  C  S  G  P  H  C  Q  R  H  V  C  L  R

       670                 690                 710
GCAGCTGCTCACTTTCTTCGAGAAGGCCGCCGAGCCCCACGCGCAGGGCCTGCTACTGTG
 Q  L  L  T  F  F  E  K  A  A  E  P  H  A  Q  G  L  L  L  C

       730                 750                 770
CCCATGTGCCCCCAACGACCGGGGCTGCGGGGAGCGCCGGCGCAACACCATCGCCCCCAA
 P  C  A  P  N  D  R  G  C  G  E  R  R  R  N  T  I  A  P  N

       790                 810                 830
CTGCGCGCTGCCGCCTGTGGCCCCCAACTGCCTGGAGCTGCGGCGCCTCTGCTTCTCCGA
 C  A  L  P  P  V  A  P  N  C  L  E  L  R  R  L  C  F  S  D

       850                 870                 890
CCCGCTTTGCAGATCACGCCTGGTGGATTTCCAGACCCACTGCCATCCCATGGACATCCT
 P  L  C  R  S  R  L  V  D  F  Q  T  H  C  H  P  M  D  I  L

       910                 930                 950
AGGAACTTGTGCAACAGAGCAGTCCAGATGTCTACGAGCATACCTGGGGCTGATTGGGAC
 G  T  C  A  T  E  Q  S  R  C  L  R  A  Y  L  G  L  I  G  T
```

FIG. 7B

```
970                 990                 1010
TGCCATGACCCCCAACTTTGTCAGCAATGTCAACACCAGTGTTGCCTTAAGCTGCACCTG
 A  M  T  P  N  F  V  S  N  V  N  T  S  V  A  L  S  C  T  C

1030                1050                1070
CCGAGGCAGTGGCAACCTGCAGGAGGAGTGTGAAATGCTGGAAGGGTTCTTCTCCCACAA
 R  G  S  G  N  L  Q  E  E  C  E  M  L  E  G  F  F  S  H  N

1090                1110                1130
CCCCTGCCTCACGGAGGCCATTGCAGCTAAGATGCGTTTTCACAGCCAACTCTTCTCCCA
 P  C  L  T  E  A  I  A  A  K  M  R  F  H  S  Q  L  F  S  Q

1150                1170                1190
GGACTGGCCACACCCTACCTTTGCTGTGATGGCACACCAGAATGAAAACCCTGCTGTGAG
 D  W  P  H  P  T  F  A  V  M  A  H  Q  N  E  N  P  A  V  R

1210                1230                1250
GCCACAGCCCTGGGTGCCCTCTCTTTTCTCCTGCACGCTTCCCTTGATTCTGCTCCTGAG
 P  Q  P  W  V  P  S  L  F  S  C  T  L  P  L  I  L  L  L  S

1270                1290                1310
CCTATGGTAGCTGGACTTCCCCAGGGCCCTCTTCCCCTCCACCACACCCAGGTGGACTTG
 L  W  *

1330                1350                1370
CAGCCCACAAGGGGTGAGGAAAGGACAGCAGCAGGAAGGAGGTGCAGTGCGCAGATGAGG

1390                1410                1430
GCACAGGAGAAGCTAAGGGTTATGACCTCCAGATCCTTACTGGTCCAGTCCTCATTCCCT

1450                1470                1490
CCACCCCATCTCCACTTCTGATTCATGCTGCCCCTCCTTGGTGGCCACAATTTAGCCATG
```

FIG. 7C

1510 1530 1550

TCATCTGGTGGTGACCAGCTCCACCAAGCCCCTTTCTGAGCCCTTCCTCTTGACTACCAG

1570 1590 1610

GATCACCAGAATCTAATAAGTTAGCCATTCTCTATTGCATTCCAGATTAGGGTTAGGGTA

1630 1650 1670

GGGAGGACTGGGTGTTCTGAGGCAGCCTAGAAAGTCATTCTCCTTTGTGAAGAAGGCTCC

1690 1710 1730

TGCCCCCTCGTCTCCTCCTCTGAGTGGAGGATGGAAAACTACTGCCTGCACTGCCCTGTC

1750 1770 1790

CCCGGATCCTGCCGAACATCTGGGCATCAGGAGCTGGAGCCTGTGGGCCTTGCTTTATTC

1810 1830 1850

CTATTATTGTCCTAAAGTCTCTCTGGGCTCTTGGATCATGATTAAACCTTTGACTTAAAA

1870

AAAAAAAAAAAAAAAAAA

FIG. 7D

```
 14 VLMLLLLLPPSPLPLAAGDPLPTESRLMNSCLQARRKCQADPTCSAAYHH  63
    :::  |.:: . |.|  :..:..:.||  .|:.|. .|  :..||. |:
  1 MFLATLYFALPLLDLLMSAEVSGGDRL..DCVKASDQCLKEQSCSTKYRT  48

 64 LDSCTSSISTPLP.SEEPSVPADCLEAAQQLRNSSLIGCMCHRRMKNQVA 112
    |  |..: .| :. ..: ....:| .| :.|:..||..|.|.| ||.: .
 49 LRQCVAGKETNFSLTSGLEAKDECRSAMEALKQKSLYNCRCKRGMKKEKN  98

113 CLDIYWTVHRARSLGNYELDVSPYE.......DTVTSKPWKMNLSKLNML 155
    || |||.:...    ||  |: ||||       |.. . |:  :: .
 99 CLRIYWSMYQS.LQGNDLLEDSPYEPVNSRLSDIFRAVPFISDVFQQVEH 147

156 KPDSDLCLKFAMLCTLNDKCDRLRKAYGEAC....SGPHCQRHVCLRQLL 201
     ..:: ||. |. |.|:|.|.:.|.|| .:|     |.. |.|: | :.|
148 ISKGNNCLDAAKACNLDDTCKKYRSAYITPCTTSMSNEVCNRRKCHKALR 197

202 TFFEKAAEPHAQGLLLCPCAPNDRGCGERRRNTIAPNCALPPVA.PNCLE 250
     ||:|.:..|. |:|:|.|  .| :|.||||.||.|.|.... . ||||.
198 QFFDKVPAKHSYGMLFCSC..RDIACTERRRQTIVPVCSYEERERPNCLS 245

251 LRRLCFSDPLCRSRLVDFQTHCHPMD.ILGTCATEQ.SRCLRAYLGLIGT 298
    |.  | .: :|||||.|| |:|:|  .  ::.| .|. . || || |||||
246 LQDSCKTNYICRSRLADFFTNCQPESRSVSNCLKENYADCLLAYSGLIGT 295

299 AMTPNFVSNVNTSVALSCTCRGSGNLQEECEMLEGFFSHNPCLTEAI... 345
    .||||:|.. . ||| .|.|..|||  |:| .: .||..|.||.:||
296 VMTPNYVDSSSLSVAPWCDCSNSGNDLEDCLKFLNFFKDNTCLKNAIQAF 345

346 ....AAKMRFH...........SQLFSQDWPHPTFAVMAHQNENPAVRPQ 380
        |.|:: :           |:: .:::: :.  : ..  ..|:  .
396 ANLQAQKLKSNVSGSTHLCLSDSDFGKDGLAGASSHITTKSMAAPPSCSL 445

381 PWVPSLFSCTLPLILLLSL 399
    ..:| |: ..|: :| :||
446 SSLPVLMLTALAALLSVSL 464
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FIG.8

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/12, C07K 14/71, A61K 38/17

A3

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Filed on 27 June 1997 (27.06.97)

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(72) Inventors; and
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(74) Agents: STEFFE, Eric, K.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005–3934 (US) et al.

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(54) Title: GDNF RECEPTORS

(57) Abstract

The present invention relates to a novel glial cell line–derived neurotrophic factor receptor beta (GDNFR–β) and novel glial cell line–derived neurotrophic factor receptor gamma 1 and 2 (GDNFR–γ1 and GDNFR–γ2). The receptors of the present invention share high homology with glial cell line–derived neurotrophic factor receptor alpha and have been named GDNFR–β, GDNFR–γ1 and GDNFR–γ2. More specifically, isolated nucleic acid molecules are provided encoding human GDNFR–β, GDNFR–γ1 and GDNFR–γ2 receptors. GDNFR–β, GDNFR–γ1 and GDNFR–γ2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of GDNFR–β, GDNFR–γ1 and GDNFR–γ2 activity. Also provided are diagnostic and therapeutic methods for disorders and diseases including Parkinson's disease, thyroid tumor, kidney failure and gut dysfunction.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/10328

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/71 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ADAMS M D ET AL: "3,400 NEW EXPRESSED SEQUENCE TAGS IDENTIFY DIVERSITY OF TRANSCRIPTSIN HUMAN BRAIN" NATURE GENETICS, vol. 4, no. 3, July 1993, pages 256-267, XP000611495 see the whole document -& DATABASE EMBL - EMEST11 Entry HSZZ55103, Acc.No. AA349976, 18 April 1997 ADAMS, M.D. ET AL.: "EST56965 Infant brain Homo sapiens cDNA 5' end." XP002072636 see the whole document ---	23

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 1998	27.11.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/10328

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	ADAMS M D ET AL: "INITIAL ASSESSMENT OF HUMAN GENE DIVERSITY AND EXPRESSION PATTERNS BASED UPON 83 MILLION NUCLEOTIDES OF CDNA SEQUENCE" NATURE, vol. 377, 28 September 1995, pages 3-17, XP002042918 see the whole document -& DATABASE EMBL - EMEST11 Entry HSZZ82801, Acc.No. AA377675, 18 April 1997 ADAMS, M.D. ET AL.: "EST90281 Synovial sarcoma Homo sapiens cDNA 5' end." XP002072637 see the whole document ---	23
A	JING S ET AL: "GDNF-INDUCED ACTIVATION OF THE RET PROTEIN TYROSINE KINASE IS MEDIATED BY GDNFR-ALPHA, A NOVEL RECEPTOR FOR GDNF" CELL, vol. 85, 28 June 1996, pages 1113-1124, XP002036435 cited in the application see the whole document ---	
P,X	BALOH, R.H. ET AL.: "TrnR2, a novel receptor that mediates neurturin and GDNF signaling through Ret." NEURON, vol. 18, May 1997, pages 793-802, XP002072635 see figure 1 ---	1-20,23
P,X	WO 97 44356 A (BIOGEN INC ;SANICOLA NADEL MICHELE (US); HESSION CATHERINE (US); C) 27 November 1997 see the whole document -----	1-23

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ternational application No.

PCT/US 98/10328

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 21 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-23,49,50

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-23,49,50

   GDNF Receptor-beta in its pro- and mature forms, the receptor with an amino acid substitution, the extracellular or transmembrane portions and epitope-bearing portions of the receptor, and nucleic acids encoding them or their complementary or hybridyzing sequences, recombinant vector comprising said nucleic acid and method for making it, recombinant host comprising said vector and method for making it, method for recombinant production of said proteins using the transformed host, antibody against GDNFR-beta and use of the receptor or its (ant)agonists in a pharmaceutical composition for the treatment of diseases associated with altered GDNFR activity.

2. Claims: 24-48

   GDNF Receptor-gamma1 in its pro- and mature forms, the receptor with an amino acid substitution, the extracellular or transmembrane portions and epitope-bearing portions of the receptor, and nucleic acids encoding them or their complementary or hybridyzing sequences, recombinant vector comprising said nucleic acid and method for making it, recombinant host comprising said vector and method for making it, method for recombinant production of said proteins using the transformed host, antibody against GDNFR-gamma1 and use of the receptor or its (ant)agonists in a pharmaceutical composition for the treatment of diseases associated with altered GDNFR activity.

3. Claims: 51-75

   GDNF Receptor-gamma2 in its pro- and mature forms, the receptor with an amino acid substitution, the extracellular or transmembrane portions and epitope-bearing portions of the receptor, and nucleic acids encoding them or their complementary or hybridyzing sequences, recombinant vector comprising said nucleic acid and method for making it, recombinant host comprising said vector and method for making it, method for recombinant production of said proteins using the transformed host, antibody against GDNFR-beta and use of the receptor or its (ant)agonists in a pharmaceutical composition for the treatment of diseases associated with altered GDNFR activity.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/10328

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9744356 A	27-11-97	AU 3472997 A	09-12-97

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